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Pacific Northwest Laboratory Annual Report for 1989 to the DOE Office of Energy Research

Part 4 Physical Sciences
April 1990

Prepared for the U.S. Department of Energy
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Pacific Northwest Laboratory
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**Pacific Northwest Laboratory
Annual Report for 1989 to the
DOE Office of Energy Research**

Part 4 Physical Sciences

L. H. Toburen, B. R. Stults,
J. A. Mahaffey, and members of
the Physical and Technological
Programs Staff

April 1990

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

Preface

This 1989 Annual Report from Pacific Northwest Laboratory (PNL) to the U.S. Department of Energy (DOE) describes research in environment, safety, and health conducted during fiscal year 1989. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of the PNL program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Assistant Secretary for Environment, Safety, and Health. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1989 Annual Report are:

Part 1: Biomedical Sciences

Program Manager:	J. F. Park	J. F. Park, Report Coordinator S. A. Kreml, Editor
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Part 2: Environmental Sciences

Program Manager:	R. E. Wildung	M. G. Hefty, Report Coordinator and Editor
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Part 3: Atmospheric Sciences

Program Manager:	C. E. Elderkin	C. E. Elderkin, Report Coordinator E. L. Owczarski, Editor
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Part 4: Physical Sciences

Program Manager:	L. H. Toburen	L. H. Toburen, Report Coordinator K. A. Parnell, Editor
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**Part 5: Environment, Safety, Health,
and Quality Assurance**

Program Managers:	L. G. Faust P. G. Doctor J. M. Selby	S. K. Ennor, Report Coordinator and Editor
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Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

Credit for this annual report goes to the many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman, editor in chief, who directed the total effort.

W. J. Bair and T. S. Tenforde
Environment, Health, and Safety
Research Program

Previous reports in this series:

Annual Report for:

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1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280, BNWL 235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4
1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5
1981	PNL-4100, Pt. 1-5
1982	PNL-4600, Pt. 1-5
1983	PNL-5000, Pt. 1-5
1984	PNL-5500, Pt. 1-5
1985	PNL-5750, Pt. 1-5
1986	PNL-6100, Pt. 1-5
1987	PNL-6500, Pt. 1-5
1988	PNL-6800, Pt. 1-5

Foreword

Part 4 of the Pacific Northwest Laboratory Annual Report for 1989 to the DOE Office of Energy Research includes those programs funded under the title "Physical and Technological Research." The Field Task Program Studies reported in this document are grouped by budget category and each Field Task proposal/agreement is introduced by an abstract that describes the projects reported in that section. These reports only briefly indicate progress made during 1989. The reader should contact the principal investigators named or examine the publications cited for more details.

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Chernobyl Database Management

The Chernobyl Database project staff continue to develop and maintain an information system composed of several databases and software tools to provide researchers with convenient access to information relating to the Chernobyl nuclear accident in April 1986. Work performed on the Chernobyl Database project during FY 1989 included acquiring radiological measurements for approximately 15,000 samples; acquiring over 2,000 bibliographic citations; acquiring over 200 publications for the database library, many from comparatively inaccessible sources; advertising the availability of resources; distributing a report entitled "Chernobyl Bibliography"; and establishing agreements regarding information exchange with staff of two Chernobyl databases in Europe. In subsequent years, we will continue to obtain and load data, enhance data standardization and quality, publicize the system, and support researcher requests for information. In addition, a database of summarized information (e.g., charts, graphs, figures, plots) will be developed to allow users convenient access to information that is not widely available.

Chernobyl Database

J. A. Mahaffey, F. Carr, Jr., and J. M. McClelland

Measurements Database

Two major data sets were added to the database of measurements in FY 1989. These sets, from the Ministry of Agriculture, Food, and Fisheries in the United Kingdom and the National Institute of Radiological Sciences in Japan, together added about 15,000 samples and 35,000 results to the database. Each sample in the database may have one or more results associated with it. Other data are in various stages of entry into the information system, including the ongoing activity of standardizing data, both newly acquired data and data entered previously. Table 1 shows the number of samples obtained from each major data set available in the system to date. The approximately 63,000 samples have provided about 92,000 measurement results.

Figures 1 and 2 show the distribution of samples in the measurements database by geographic location. Figure 3 shows the distribution of samples by media. The figure indicates that the Chernobyl Database is dominated by air samples and also has large numbers of samples in food sources, such as samples of animals and vegetation. Figures 1 through 3 are based on measurement samples. For elements with over 1000 results in the database, Figure 4 shows the number of measurement results and indicates that most results are for cesium and iodine.

TABLE 1. Distribution of Samples by Data Set Source

Data Set Source	Number of Samples
Brookhaven National Laboratory	38
Commissariat A L'Energie Atomique (France)	20,463
Department of Social and Health Services (Washington State)	454
EG&G Idaho, Inc.	7,067
Ministry of Agriculture, Food, and Fisheries (United Kingdom)	8,535
National Institute of Radiological Sciences (Japan)	6,841
Stone and Webster	9,407
U.S. Environmental Protection Agency (Domestic)	6,301
U.S. Environmental Protection Agency (Foreign)	3,806
Total	62,912

Much of the data currently being obtained for the database are summarized information. Based on written communications to authors of publications containing Chernobyl-related information, we learned that data are often unavailable except in a summarized form. Thus, a database of summarized information, such as charts, graphs, figures, and plots, will be developed to allow users convenient access to information that is not widely available. Emphasis will be on including summarized information from sources other than open literature publications.

Bibliographic Database/Library

A collection of publications containing information about the Chernobyl accident is being assembled

FIGURE 1. Distribution of Radiological Measurement Samples by Country of Origin

FIGURE 2. Distribution of Radiological Measurement Samples in the United States

FIGURE 3. Distribution of Radiological Measurement Samples by Media

FIGURE 4. Distribution of Radiological Measurement Results for Elements with Over 1000 Results

to form a Chernobyl library. Information pertaining to documents and the organizations that published them has been incorporated into the bibliographic database. Approximately 330 publications reside in the library and computerized bibliography, 200 collected this past year. Emphasis is on obtaining comparatively inaccessible documents.

In addition, a literature search of the U.S. Department of Energy's Energy Database identified 2023 citations that could be standardized and loaded into the bibliographic database. If an abstract were available from either a document or an electronic source, it was included in the bibliographic database. The number of citations that are books, journal articles, and reports by country is given in Table 2; the greatest number of citations are from the Federal Republic of Germany, the United Kingdom, and the United States. Other countries with a large number of citations are Austria, Italy, Japan, Netherlands, Switzerland, and the USSR.

The bibliography was published and distributed as a document during this past year, and will soon be available electronically via a run-time version of the data management software PARADOX. This will allow users local access to the information on their own workstations for interrogation and reformatting.

Acquiring Additional Information/Publicizing Availability of Resource

Our approach to system design and development, content of current databases, and system functionality was presented at the annual meeting of the Society for Risk Analysis. In addition, data were solicited and resource availability was advertised in the *Health Physics Newsletter*, *Amstat News*, and *Risk Newsletter*.

Throughout the year, approximately 400 letters soliciting data and database users were written to various organizations and individuals in the following countries (those countries that replied are in bold type): Algeria, **Australia**, **Austria**, Bangladesh, **Belgium**, Brazil, Bulgaria, Cameroun, **Canada**, Congo, Cyprus, **Czechoslovakia**,

TABLE 2. Summary of Entries in Bibliographic Database by Type of Publication

Country	Number of Citations		
	Books	Journals	Reports
Africa	0	3	2
Australia	0	17	2
Austria	26	38	33
Belgium	0	10	6
Canada	1	1	0
China	0	4	1
Czechoslovakia	0	10	1
Denmark	1	0	18
Finland	1	0	23
France	4	9	37
Germany, D.R.	0	7	4
Germany, F.R.	156	266	76
Greece	0	0	7
Hungary	0	6	4
India	1	2	1
Israel	1	0	3
Italy	11	27	17
Japan	9	45	8
Netherlands	22	31	27
Norway	3	3	15
Pakistan	0	0	1
Philippines	0	0	3
Poland	0	0	1
South America	0	4	4
Spain	0	1	1
Sweden	6	3	40
Switzerland	17	96	3
Thailand	0	1	0
United Kingdom	146	340	21
United States	48	112	107
USSR	9	32	7
Yugoslavia	20	0	0
Total	482	1068	473

Denmark, England, Ethiopia, Finland, France, **Federal Republic of Germany**, German Democratic Republic, Ghana, Greece, **Guatemala**, Hungary, Iceland, India, Iran, **Ireland**, **Italy**, Ivory Coast, **Japan**, Jordan, Kenya, Kuwait, Liberia, Luxembourg, Madagascar, Mali, Mauritania, Mexico, Nepal, **Netherlands**, Niger, Nigeria, **Norway**, Pakistan, Panama, **People's Republic of China**, Peru, Philippines, Poland, **Portugal**, **Romania**, Samoa, **Scotland**, Senegal, **Spain**, Sudan, **Sweden**, **Switzerland**, Thailand, Tunisia, **Turkey**, **United States**, USSR, **Uruguay**, **Wales**, **Yugoslavia**, Zaire, and **Zimbabwe**. Replies were received from over 35% of the correspondents and continue to be received.

As a result of the presentation and correspondence, 15 additional requests for information from the system were received. Of these, five requests resulted in on-line user access to the databases. This brings the total number of users to 39, about 60% of which are from the United States, and the remainder are located throughout the world.

Collaborations with Other Chernobyl Databases

Agreements were reached with the Joint Research Center at Ispra, Italy, regarding their Radiological Environmental Monitoring (REM) database. This is the resource containing data from the 12 member countries of the European Community, with additional data from Austria. We agreed to 1) regularly exchange information on the content of our respective databases, 2) refer users making requests from either source to similar information available from the other database, and 3) avoid duplication of effort to the extent possible in any

aspect of data collection, evaluation, organization, or computerization, and in any aspect of obtaining or computerizing reference material or citations. We are developing software for use in exchanging and updating information on the content of our respective databases; we will exchange the number of records by country, media, nuclide (^{134}Ce , ^{137}Ce , and ^{131}I by isotope, and the others by element), whether the data were collected pre- or post-Chernobyl, and the source of information (for sources providing 50 or more records).

In addition, ties were established with the Nordic Chernobyl Database (NCDB). This database differs substantially from the other two in that member countries are conducting an intercalibration exercise to ensure that measurement data are consistent. Participants include twelve laboratories in Denmark (where the database is located), Finland, Faroe Islands, Iceland, Norway, and Sweden.

Determination of Radon Exposure

The goal of this project is to determine the correlation between radon exposure and the ratio of ^{210}Pb to ^{208}Pb in the body. This requires development of a sensitive analytical procedure capable of measuring extremely small quantities of ^{210}Pb in the presence of about 10^{10} greater amounts of stable Pb isotopes. The method being developed uses high-resolution continuous wave (cw) laser ionization techniques coupled with mass spectrometry to provide the requisite selectivity and a sensitivity sufficient for dealing with small samples. Once analytical capabilities are established, the research will be aimed at determining the correlation between radon exposure and the ^{210}Pb : ^{208}Pb ratio in various tissues and how specific factors such as diet influence that correlation. Also, the method will be used for determining long-term integrated radon levels in homes and the environment.

Selectivity and Sensitivity in the Detection of ^{210}Pb

B. A. Bushaw, W. L. Glab, G. K. Gerke, and J. T. Munley

Studies in the first year of this program investigated the overall photoionization scheme with stable Pb isotopes, determined energies of the Rydberg states with moderate-resolution pulsed lasers, measured isotope shifts and selectivity in the first resonant excitation, and obtained preliminary measurements of isotope shifts and selectivity in double-resonance excitation to the Rydberg states with high resolution (Bushaw et al. 1989). This year, the high-resolution, double-resonance studies have been continued and are systematically determining the isotope shifts for a variety of Rydberg states in the energy region of interest.

The state with the most favorable shifts for the selective excitation of ^{210}Pb has been determined, and studies have progressed to actual measurements on ^{210}Pb . Considerable attention has been given to improving instrumental sensitivity by optimizing sample evolution, excitation, and detection geometries. Also, the development of chemical preparation procedures for "real-world" samples has begun.

The initial task for this program, the characterization of the double-resonance ionization process for stable Pb isotopes, has been completed. Isotope shifts (and hyperfine structure for ^{207}Pb) were measured in a number of additional Rydberg states; the results are summarized in Table 1. The known isotope shift for ^{210}Pb in the first resonance (Thompson et al. 1983) and proportionality

TABLE 1. Measured Optical Shifts for the Stable Isotopes of Pb (in MHz with respect to ^{208}Pb) in Various Rydberg States. The calculated shift for ^{210}Pb in the final column is based on the measured even isotope shifts and proportionality relations.

Rydberg State, J	Optical Shifts, MHz			
	^{204}Pb	^{206}Pb	^{207}Pb (a)	^{210}Pb
6p12f (1/2[5/2]), 2	-2880	-1518	-3711 (5/2), +1822 (3/2)	+2712
6p15p(1/2,1/2), 0	-2762	-1468	+1883	+2621
6p15p(1/2,3/2), 1	-2907	-1535	-2467 (3/2), +2271 (1/2)	+2740
6p16p(1/2,3/2), 1	-2915	-1541	-2621 (3/2), +2277 (1/2)	+2749
6p16p(1/2,3/2), 2	-2814	-1487	-3183 (3/2), +545 (5/2)	+2653 ^(b)

(a) For ^{207}Pb in states with $J \neq 0$, the line is split into a hyperfine doublet and the position of both components are given with the hyperfine quantum number F in parentheses.

(b) State selected for analytical measurements of ^{210}Pb . The actual measured shift is +2632 MHz.

relations for the even isotopes (Bushaw 1989) were then used to calculate the expected value of the ^{210}Pb shift in the Rydberg states. The best state $[6p16p(1/2,3/2), J = 2]$, with a calculated ^{210}Pb shift of 2653 MHz with respect to ^{208}Pb , was selected for further studies. Actual measurements on ^{210}Pb used a National Institute of Standards and Technology reference solution that contained ^{210}Pb at a concentration of 4 ppm with respect to the stable Pb isotopes. The results are summarized as follows.

Spectroscopic studies were carried out with sample loadings of 0.8 pg ^{210}Pb and the graphite furnace held at constant temperature for slow sample evolution. The first laser was held fixed at the known ^{210}Pb resonance; the second laser was scanned to determine the isotope shift in the Rydberg state. The ^{210}Pb isotope shift was found to be +2632 MHz from ^{208}Pb (agreeing with the a priori calculation to within 0.8%), placing it 2087 MHz from the nearest interference ($F = 5/2$ of ^{207}Pb), and the calculated optical isotopic selectivity was 10^9 .

Analytical studies were carried out by ramping the furnace temperature to evolve all of the sample over a period of about 15 min. The second laser was tuned 100 MHz off resonance for 30 s of every minute to determine the background. An example of this type of measurement is shown in Figure 1. In this measurement, the mass spectrometer was not used so that the selectivity of the optical processes could be evaluated directly. Integration of the signal and background out to the point labeled A yields a signal-to-background noise ratio of ~ 500 . At higher temperatures, beyond point A, a background signal not due to Pb appears. This is thought to be due to K in the sample, which has an ionization potential low enough that it may be directly photoionized by one photon from the first dye laser in the Pb excitation scheme. This K background will be easily removed when the mass spectrometer is used. These studies showed that optical selectivity for ^{210}Pb versus other Pb isotopes was greater than 10^8 ; however, sample size and dynamic range were not sufficient to determine the actual limit. Detection limits are currently estimated to be slightly less than 1 fg (0.08 pCi); however, with the inclusion of the mass spectrometer for removal of

FIGURE 1. Analytical Measurement on a Sample Containing 400 fg ^{210}Pb (4 ppm with respect to other Pb isotopes). Observed count rate is shown as a function of time as the graphite furnace temperature is ramped to evolve the sample. The square wave structure is due to switching the second laser on and off the ^{210}Pb resonance for background determination. The nonresonant background that grows in after point A is probably due to K, as described in the text.

the K background, as well as further improvements in the laser excitation geometry, the detection limits should improve by another one to two orders of magnitude. Also, the mass spectrometer will boost the overall Pb isotope selectivity into the range of 10^{13} . This should be sufficient for background level measurements on 1-g hair or bone samples, which are expected to contain ~ 1 fg of ^{210}Pb .

The development of chemical procedures for the treatment of hair samples was begun in this year. The procedure used begins with wet ashing with nitric acid and hydrogen peroxide. The remaining residue is brought up to a 0.1-mL volume with a 5% citric acid solution. The result is a sample solution suitable for loading into the graphite furnace in a matrix that gives good atomization yields. This procedure has been tested by measuring the natural Pb content in a small aliquot corresponding to a 2-mg hair sample. The measured value of 30 ppm is consistent with the "accepted"

value of 10 to 100 ppm. Measurements of ^{210}Pb may require an additional ion exchange preconcentration step so that all the Pb from a 1-g hair sample may be loaded in the furnace at one time; this will be addressed as part of the ongoing research.

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Johnson, B. A., G. L. Gault, and T. J. Winkler. 1983. A study of the effects of the 1982-83 drought on the reproductive success of the American kestrel (*Falco tinnunculus*) in the Great Plains. *Journal of Wildlife Management* 47: 1-10.

Johnson, B. A., G. L. Gault, and T. J. Winkler. 1984. A study of the effects of the 1983-84 drought on the reproductive success of the American kestrel (*Falco tinnunculus*) in the Great Plains. *Journal of Wildlife Management* 48: 1-10.

Johnson, B. A., G. L. Gault, and T. J. Winkler. 1985. A study of the effects of the 1984-85 drought on the reproductive success of the American kestrel (*Falco tinnunculus*) in the Great Plains. *Journal of Wildlife Management* 49: 1-10.

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Johnson, B. A. 1983. Reproductive success of the American kestrel (*Falco tinnunculus*) in the Great Plains. *Journal of Wildlife Management* 47: 1-10.

DNA Adducts as Indicators of Health Risks

The objective of this program is to develop specific analytical methods for the determination of adducts formed in mammals by the reaction of carcinogenic compounds with cellular DNA. DNA adducts are closely associated with the formation of cancerous cells, and their concentrations are thought to be related to the amount of exposure to carcinogenic chemicals. This program is developing mass spectrometric methods for analysis of adducts that may be applied toward health studies of human exposure to carcinogens.

Determination of Adducts of Polycyclic Aromatic Hydrocarbons to DNA

*R. M. Bean, B. L. Thomas, D. A. Dankovic,
D. B. Mann, and D. L. Springer*

Metabolites of carcinogenic organic compounds have the ability to bond with DNA to form DNA adducts. These species are retained for relatively long periods of time in the body and are thought to be associated with the formation of cancer. Analysis of DNA for adducts may therefore provide an estimate of individual exposure to carcinogens. Methods currently being used for analysis of DNA adducts have been reviewed by Wogan and Gorelick (1985). In general, the currently available methods suffer either from a lack of sufficient sensitivity for environmental screening or from a lack of qualitative specificity. Although data on humans are sparse, allowing for initial rapid decay after environmental exposure, adduct levels of 0.1 to 0.01 ng/mg of DNA may be expected. The objective of this project is to develop methods for the analysis of DNA adducts that will permit identification and quantitation of adducted polycyclic aromatic hydrocarbon (PAH) metabolites at environmental levels. Included within this objective is the preparation and characterization of DNA adducts to be used as analytical standards.

Biological Preparation of Adducts

A method for preparation of a variety of hydrocarbon-derived DNA adducts has been developed by Dankovick et al. (1989) and appears to be a satisfactory method for obtaining experimental quantities of a variety of PAH adducts. Previously, the availability of adducts in quantities suitable for analytical characterization has been a serious limitation in research directed toward adduct analysis. Adduct standards prepared by chemical synthesis require a previous knowledge of adduct structure and chirality, but the

hepatocyte method requires no such foreknowledge. Preparations that use microsomal ("S-9") fractions to afford the adduction between hydrocarbon and DNA produce highly polar products and do not produce adducts in the microgram quantities required for analytical study.

The procedure uses freshly isolated rat hepatocytes, which are incubated together with calf thymus DNA and the PAH to be adducted. The products from this *in vitro* hepatocyte method seem to very closely resemble adduct distributions found from *in vivo* studies of adducts formed from the topical application of PAH to mouse skin. For the first time, research quantities of many different adducts can be prepared from adducting hydrocarbons and pure DNA. Table 1 lists the adducts prepared and the quantities realized from the preparation.

The adducts have all been prepared using radio-labeled hydrocarbons so that the adduction efficiency could be monitored and so that further analytical characterization could be facilitated. The quantities given in Table 1 may seem small, but they far exceed quantities thus far reported for adducts formed from biological systems; furthermore, the method is capable of producing larger amounts than given above. The hepatocyte method appears to be a substantial contribution to activities required to develop methods for analysis of adducts at environmental levels. Requests have

TABLE 1. List of Adducts Prepared By the Hepatocyte Method

Adduct	Quantity Prepared
Dibenz[a,h]anthracene	200 ng
7, 12-Dimethylbenzanthracene	110 ng
BaP	5 μ g
Fluoranthene	1.5 μ g
Benz[a]anthracene	1.1 μ g

been received from researchers in other national laboratories for samples of hepatocyte-prepared adduct material. Additional efforts are needed to establish reaction conditions that are applicable to a wide variety of hydrocarbon substrates. For example, since the rate of metabolism of each hydrocarbon by the hepatocyte system is different, the incubation conditions require preliminary experiments before microgram-scale experiments can be successfully conducted. Thus, tools for rapid metabolic screening are a needed adjunct to the methodology. Also in need of further research are ways to more efficiently use the hepatocyte system and to further scale up the size of the adduction system.

Characterizing Hepatocyte-Prepared Adduct Material

Exploitation of the hepatocyte method for preparation of hydrocarbon-DNA adducts requires a suite of analytical tools for analytical characterization. The characterization studies, to be practical, should be applicable to samples in approximately 100-ng quantities. Liquid chromatography (LC) has been used as a tool for ensuring that the preparation procedure has resulted in the "right" adduct by comparing adduct chromatograms with those reported in the literature. With the fluoranthene adduct, liquid radiochromatography was essential for verification of the identity since the adduct was not fluorescent, and thus could not be characterized by its fluorescence spectrum. Modern LC instrumentation has the capability for obtaining complete spectroscopic scans of separated analytes. Ultraviolet spectra are obtained through the use of a diode array detector, which can determine all wavelengths simultaneously; fluorescence detection is accomplished through a "stop-flow" technique, where solvent flow is stopped while the analyte is scanned with the fluorescence detector. Stop-flow fluorescence scanning with the present LC instrumentation is not sensitive enough to be applicable to intact benzo[a]pyrene (BaP) adduct (hydrocarbon metabolite attached to DNA base) at the 100-ng level because of loss in sensitivity by the detector while scanning and internal quenching in the adduct. However, after the hydrocarbon metabolite is freed from the DNA base by hydrolysis, the fluorescence response is sufficient to obtain a fluorescence spectrum at the 100-ng level.

Acid hydrolysis of the adduct to free the hydrocarbon metabolite from the DNA, followed by LC separation of the metabolite and further characterization of the metabolite by spectral and mass spectrometric methods appears to be a feasible approach to analyzing the adducts prepared in the laboratory. Considerable effort has been spent applying this approach to the fluoranthene adduct. However, for this adduct, satisfactory hydrolysis conditions that result in the expected tetrahydrotetrol metabolite have not been found. The principal product from the hydrolysis conditions is a very nonpolar component that is not recoverable because of its relatively high volatility. Attempts to liberate a tetrol using a variety of hydrolysis conditions have not been successful. This indicates that the proposed analytical scheme [hydrolysis to tetrol, derivatization, and detection by mass spectroscopy (MS)] may not be applicable to all DNA adducts formed from aromatic hydrocarbon metabolites.

Another potentially powerful tool for characterization of hepatocyte-prepared adducts is LC/MS. Critical to the success of LC/MS methods is an interface that maximizes analyte signal while efficiently removing most of the liquid solvent used for the separation. A new "particle beam" interface is being evaluated for PNL's LC/MS system that has promise for producing more spectral detail at much greater sensitivity than current thermospray interfaces. The new particle beam interface produces complete spectral patterns for many compounds at the 100-ng level. Excellent spectral data have been obtained for 100 ng of underivatized BaP tetrahydrotetrol (Figure 1). The spectrum shows evidence for a reverse Diels-Alder cleavage that has previously been demonstrated for permethyl and persilyl derivatives of the tetrol (Chess et al. 1988). When a 200-ng sample of intact BaP-guanosine adduct was subjected to this type of LC/MS analysis, no signal was obtained. This result, although disappointing, was probably associated with the design of the transfer lines and can be rectified with further experimentation.

GC/MS Analysis of DNA-Adduct Metabolites

The trimethylsilyl derivative of BaP tetrol undergoes cleavage to yield a fragment ion (404 amu) that has very little interference in the

FIGURE 1. Mass Spectrum Obtained from Approximately 100 ng of Underivatized BaP Tetrahydrodiol Using a Hewlett-Packard LC/MS System Equipped with a Particle Beam Interface

single-ion chromatogram. The derivative is more readily and reliably prepared than the corresponding methyl ethers (Chess et al. 1988), and unoptimized detection levels are at the 1-pg level. The use of silylation to prepare a stable, readily detectable tetrol derivative for gas chromatography-MS (GC/MS) detection has been a very favorable development for this program. With a reliable derivatization, BaP tetrols can routinely be detected with an unoptimized mass selective detector. One of the single biggest obstacles to the routine analysis at the 0.1- to 1-pg (0.3- to 3-fmol) level is the problem of quantitative transfer of analyte in 1- to 2-mL solvent to a 1- μ L volume in a syringe without substantial mechanical losses or contamination.

At the annual U.S. Department of Energy Life Sciences Contractor's Conference held on November 17 and 18, 1989, in Albuquerque, New Mexico, Dr. Allen Tischler, from Lawrence Berkeley Laboratory, suggested the possibility of analyzing for DNA adducts by releasing the hydrocarbon moiety through oxidative hydrolysis (Figure 2). If it were possible to release the adducting hydrocarbon moiety in a form permitting direct injection into the GC/MS without first derivatizing, considerable efficiency in the analysis could be realized. Periodic acid has been shown to oxidize BaP directly to 7,8-diformyl pyrene at room temperature (Figure 2). These initial experiments have shown that

FIGURE 2. Reaction Scheme Showing the Oxidation of BaP Tetrahydrodiol to the Corresponding Dialdehyde with Periodic Acid and the Proposed Sequence to Form the Dialdehyde from the DNA Adduct

the diformyl compound is readily recovered from the reaction of BaP tetrol at the 100-ng level by solid-phase absorption and GC/MS analysis. Prominent ions at $m/e = 258, 229$, and 201 readily serve to characterize the product as the diformyl product. Some time has been spent investigating whether the oxidation reaction can be extended to the oxidation of the intact BaP nucleoside adduct. The objective of these experiments is to develop a procedure to treat adducted DNA with periodic acid, isolate the diformyl product by reverse-phase adsorption, and immediately analyze by GC/MS. Attempts to accomplish this chemistry using synthetically prepared BaP-adducted DNA have thus far not resulted in detectable quantities of the diformyl product.

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Figure 1. Effect of temperature on the rate of polymerization. The rate of polymerization was measured at various temperatures in the range 100–150°C. The concentration of the monomer was 0.5 M and the concentration of the initiator was 0.01 M.

The effect of temperature on the rate of polymerization was studied in the range 100–150°C. The rate of polymerization was measured at various temperatures in the range 100–150°C. The concentration of the monomer was 0.5 M and the concentration of the initiator was 0.01 M. The rate of polymerization was found to increase sharply with increasing temperature in the range 100–110°C and then to level off at higher temperatures. This behavior is characteristic of a reaction which is controlled by the rate of initiation at low temperatures and by the rate of propagation at high temperatures. The activation energy for the initiation step was found to be 15 kJ/mol and for the propagation step 5 kJ/mol.

At the same time, the effect of the concentration of the monomer on the rate of polymerization was studied. The rate of polymerization was measured at various concentrations of the monomer in the range 0.1–1.0 M. The concentration of the initiator was kept constant at 0.01 M. The rate of polymerization was found to increase with increasing concentration of the monomer. This behavior is characteristic of a reaction which is controlled by the rate of propagation. The order of reaction with respect to the monomer was found to be 1.0.

Figure 2. Effect of monomer concentration on the rate of polymerization. The rate of polymerization was measured at various concentrations of the monomer in the range 0.1–0.5 M. The concentration of the initiator was 0.01 M.

The effect of monomer concentration on the rate of polymerization was studied in the range 0.1–0.5 M. The rate of polymerization was measured at various concentrations of the monomer in the range 0.1–0.5 M. The concentration of the initiator was kept constant at 0.01 M. The rate of polymerization was found to increase linearly with increasing concentration of the monomer. This behavior is characteristic of a reaction which is controlled by the rate of propagation. The order of reaction with respect to the monomer was found to be 1.0.

At the same time, the effect of the concentration of the initiator on the rate of polymerization was studied. The rate of polymerization was measured at various concentrations of the initiator in the range 0.001–0.01 M. The concentration of the monomer was kept constant at 0.5 M. The rate of polymerization was found to increase with increasing concentration of the initiator. This behavior is characteristic of a reaction which is controlled by the rate of initiation. The order of reaction with respect to the initiator was found to be 0.5.

Biological Effectiveness of Radon Alpha Particles

The consequences of exposing cells to one or two alpha particles from radon radioactive decay products, relative to the effect of exposure to the large number of electron tracks, for equal doses is a major concern in establishing exposure limits for radon. The time between the alpha particle events also seems to influence the probability of the cell being transformed, just as the dose rate influences the extent of life shortening in animals exposed to radon. In order to study the effects of low doses of radiation and to test alternative biophysical models of the dose-rate effect, an irradiation system has been developed that will control the position and number of particles irradiating individual cells.

Alpha Particle Microbeam Irradiation System

L. A. Braby and W. D. Reece

Because high linear energy transfer (LET) radiations deliver a relatively large dose when they interact with a cell, the probability of an individual cell experiencing an interaction is low at the doses that are relevant to radiation protection. At the relatively large dose of 0.5 Gy, individual cells of a 10T1/2 culture will be traversed by an average of two alpha particles. However, each charged particle track is independent of each other track. Statistically only 27% of the cells in a population exposed to 0.5 Gy actually receive two events. Nearly 40% receive no event or only one event, and 33% receive three or more events. Environmental exposures generally occur at a low dose rate, but even at a fixed dose rate individual cells experience a wide range of times between charged particle events. If the irradiation extends over a period of time, t , and exactly two events occur in a cell, then the time between the events can be as large as t , but the most probable value is 0. Figure 1 shows the probability density for the time between events when exactly three occur. It is obviously difficult to unfold the response of cells to specific ionizing radiation events from the broad range of interactions and biological responses that occur in biological experiments utilizing randomly distributed radionuclides or charged particle tracks.

The effects of individual charged particle events and combinations of events can be investigated by limiting the area irradiated to a small part of a cell and by using a counter and shutter to limit irradiation to a specified number of particles. An accelerator beam line with a collimator and scintillation detector to perform this type of irradiation

FIGURE 1. The Probability Density of Time Between Events When Exactly Three Events Occur. The density for all pairs of events is the same for any number of events occurring during the interval T .

was described last year (Braby and Reece 1989, pp. 21-23). Some of the components of that system were much harder to obtain than expected and the collimator and particle detector were just completed at the end of fiscal year 1989.

The beam line, collimator, and cell-positioning microscope (set up for preliminary testing of the collimator and vacuum system) are shown in Figure 2. The beam exits the vacuum system through a 2- μ m-thick scintillator in the plane of the base of the microscope and stops in the cell being irradiated or in the tissue culture medium covering the cell. A photomultiplier tube is used to detect the scintillation light collected by the microscope objective. This detection system has been tested with alpha particles from a radioactive source and the microscope optics. The thin scintillator has low light output for energy deposited by alpha particles, but by using an objective lens with a numerical aperture of 0.65, several electrons are emitted by the photocathode for each event. Using a

FIGURE 2. Single Particle Irradiation System Set Up for Preliminary Vacuum and Optics Tests

cooled photomultiplier, these events can easily be distinguished from the noise, and the probability of not detecting an event should be very low. Detection of this photon signal requires a light-free environment for the scintillation film and objective lens. This has been accomplished by building the bottom half of the microscope into a large light-tight box that holds the cell-positioning stage as well as the optical components.

The mechanical shutter, which was planned to control the number of particles per dose, proved to be slower than expected, on the order of 0.1 s. Use of this slow shutter would require a lower beam current than planned and would make it difficult to control the accelerator energy and focus, as well as require more time than desired to make an irradiation. As a result, a piezoelectric translator has been substituted for the mechanical shutter.

The advantage is that it is very fast, requiring less than 0.001 s to shut off the beam; the disadvantage is that the travel is very short, so that the shutter has to be aligned with the rest of the collimator.

A personal computer will control the actual operation of the irradiation system. It will receive information from the image-processing system on the position of the target to be irradiated relative to the collimator and will control the operation of the positioning stage, the two shutters in the optical system, the beam shutter, the microscope focus, the image routing in the microscope (to video camera or photomultiplier), and possibly the microscope magnification factor. The hardware to control the motors and shutters has been interfaced to the computer along with the cell-positioning stage, and the software to position targets has been tested.

As soon as the collimator has been aligned, testing will begin on the reliability of the single-particle

detection system and the effect of scatter on the actual position of charged particle events. The initial experiment will use track etch film techniques, developed in the Dosimetry Technology Section, to measure the relative positions of actual events. A film will be mounted on the positioning stage over the collimator, and a single alpha particle track will be requested at each stop as the stage scans a 1-mm-square area in 20- μ m steps. After etching, the distribution of distances between etch pits, the frequency of multiple pits at a stop, and the frequency of unirradiated stops (the effect of noise in the photomultiplier system) will be measured.

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Biological Behavior and Dosimetry

The purpose of this project is to interpret and apply new information from the Radon Research Program and from other sources and to review and update all aspects of the dosimetry of Rn, Tn, and their radioactive decay products that are relevant to assessing human exposure and health effects. The scope of the review includes information needed to 1) compare doses between underground mines and indoor environments (for extrapolating occupational epidemiological data to the general public); 2) evaluate doses for children and infants; 3) compare doses from Rn and Tn decay products; 4) evaluate doses to red bone marrow and other body organs and their age dependence; 5) evaluate doses to the fetus; 6) evaluate doses to laboratory animals used in health effects studies; and 7) evaluate doses to cells exposed in vitro to Rn and its decay products. Best current estimates of conversion coefficients between exposure quantities and doses and their sensitivities to environmental and biological factors are being derived.

Behavior and Dosimetry of Radon

A. C. James, M. J. Egan,^(a) W. Nixon,
D. L. Swift,^(b) and T. E. Hui^(c)

The Radon Research Program is addressing the following factors that are crucial in relating human exposure to a biologically effective dose: the nature and size distribution of the "unattached" and "attached" components of Rn decay product activity in indoor air; the filtration efficiency of the nose and mouth for unattached decay products (and its variation with subject age and breathing rate); aerosol deposition behavior in the bronchial tree; the identification of sensitive target cells; and, the morphometry and cellular composition of bronchial epithelium. PNL has incorporated the results of these studies into a new model of lung deposition and bronchial dose for male and female adults, and for children exposed to Rn (or Tn) decay products.

Morphometry of Bronchial Epithelium and Target Cells

Bowden and Baldwin (1989) and Mercer et al. (1989) have conducted comprehensive studies of the thickness of epithelial tissue in the human bronchi and bronchioles, and of the distance of basal and secretory cell nuclei from the epithelial surface. The variability in thickness of bronchial epithelium is found to be significantly less than that indicated by the single preliminary study of Gastineau et al. (1972). The impact of these new

data on the estimate of average dose received by target cell nuclei in bronchial and bronchiolar epithelia from each α -decay of ^{218}Po or ^{214}Po per unit area of airway surface is shown in Table 1.

The National Council on Radiation Protection and Measurements (NCRP 1984) evaluated the dose to shallow basal cells. These were assumed to lie at 22 μm below the epithelial surface in segmental bronchi, and the Rn decay products were assumed to be mixed in a 15- μm -thick layer of mucus. The dose conversion coefficients implied by the Gastineau et al. (1972) data, for basal and secretory cell nuclei in the bronchi and for secretory cell nuclei in the bronchioles (in which there are no basal cells), were calculated by James (1988). According to these data, basal cells are distributed in the bronchi over a range of depths, with an average value of approximately 50 μm . Secretory cell nuclei were assumed to occur at all depths, from the epithelial surface through the full thickness of the epithelium. The lining layer of mucus was assumed to be 14 μm thick in the bronchi and 10 μm in the bronchioles. The average thickness of bronchiolar epithelium reported by Gastineau et al. (1972) is approximately 15 μm . This is consistent with the new and more comprehensive data of Bowden and Baldwin (1989) and Mercer et al. (1989).

For the bronchi, Bowden and Baldwin (1989) reported an average thickness of approximately 30 μm . However, in their morphometric study for the Radon Research Program, Mercer et al. (1989) reported a significantly thicker epithelium, averaging approximately 55 μm . The use of vascular perfusion of fixative in this work and meticulous sample preparation for electron microscopy enabled the thickness and composition of the

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TABLE 1. Calculated Mean Doses to Bronchial and Bronchiolar Target Cell Nuclei (in nGy) from 1 α -Decay of ^{218}Po or ^{214}Po per cm^2 of Airway Surface

	Location of Rn Decay Products				Source of Data
	Mucus		Epithelium		
	^{218}Po	^{214}Po	^{218}Po	^{214}Po	
<u>Target Cells in Bronchi</u>					
Shallow Basal	75	140	—	—	NCRP (1984)
Basal	14	47	100	110	Gastineau et al. (1972)
Secretory	76	125	110	130	
Basal	90	150	155	180	Bowden and Baldwin (1989)
Secretory + Basal	130	180	175	195	
Basal	8	70	120	130	Mercer et al. (1989)
Secretory + Basal	65	125	125	140	
<u>Target Cells in Bronchioles</u>					
Secretory	190	220	215	235	Gastineau et al. (1972)
	210	230	235	250	Bowden and Baldwin (1989)
	235	240	235	250	Mercer et al. (1989)

mucous layer to also be studied; this was found to be significantly thinner than previously assumed. In the bronchi, a gelatinous layer approximately 2 μm thick is carried on the tips of cilia that are immersed in a watery sol layer about 6 μm thick. In the bronchioles, the mucous gel is discontinuous and even thinner. This is carried on a sol layer only about 2 μm thick.

These new estimates of mucous thickness were used with the epithelial dimensions measured by Bowden and Baldwin (1989) and Mercer et al. (1989) to calculate the doses to target cell nuclei in the bronchi and bronchioles that are shown in Table 1. For the bronchi, the results of Mercer et al. confirm the earlier conclusions drawn from Gastineau et al. (1972) that the dose averaged over the whole population of secretory and basal cells from Rn decays in mucus is significantly higher than that to basal cells alone. The doses to basal cell nuclei are comparable to those received by secretory cells if the Rn decays occur in the epithelium itself. Dose conversion coefficients for the bronchioles are generally higher than for the bronchi and are independent of the location of the decays.

To formulate the dosimetric model, PNL has adopted the morphometric data of Mercer et al. (1989) and has assumed that the critical carcinogenic doses are those averaged over the whole population of secretory and basal cell nuclei in the bronchi and secretory cell nuclei in the bronchioles. The epithelial thickness can be assumed to be the same in children as in adults (Gehr 1987).

Age-Dependent Bronchial Deposition and Dose

PNL has updated the theoretical model of aerosol transport and deposition in the lung that was developed by Egan and Nixon (1985). Transport and deposition within the lung airways is represented by:

$$\frac{\delta}{\delta t} (A_T c) = - \frac{\delta}{\delta x} (A_A u c) + \frac{\delta}{\delta x} (A_A D \frac{\delta c}{\delta x}) - L \quad (1)$$

In this model, A_A is the cross-sectional area for aerosol transport, summed over all airways at distance x from the entrance of the trachea. A_T represents the total cross-sectional area in the

lung periphery and includes the additional volume associated with alveoli. The aerosol concentration, c , is governed by the mean convective flow velocity, u , the effective axial diffusion coefficient, D , and the deposition rate per unit length, L . All variables are functions of time, t , and distance, x . Equation (1) is solved numerically for $c(x,t)$ over several breaths, until ventilation and deposition rates reach equilibrium.

Deposition is evaluated as being due to the combined effects of Brownian diffusion, gravitational settling, and inertial impaction. The modeling for contributions to $L(x,t)$ from the deposition processes of inertial impaction and diffusion was based on an appraisal of experimental data obtained with hollow bronchial casts of human lung (Gurman et al. 1984; Cohen 1987). PNL used the composite model of airway dimensions that was derived by James (1988) [by averaging the dimensions reported by several authors, together with the model of the pulmonary acinus developed by Hansen et al. (1975)] to evaluate the cross-sectional area terms (A_A , A_T) in Equation (1), and to evaluate the deposition term, L , for individual airways.

Age and sex dependence of lung deposition were evaluated by scaling the dimensions of the reference lung model for the adult male (Egan et al. 1989). It was assumed that airway dimensions in the adult female lung were scaled from those of the male according to the difference in functional residual capacity (FRC). The structure of the lung in children is completely developed by 2 years of age (Gehr 1987), so that the dimensions of the pulmonary acinus can be assumed to scale with the FRC. In children, the dimensions of the bronchi (defined in the lung model as the first eight airway generations) were assumed to scale with body height in the manner described by Phalen et al. (1985). The dimensions of the bronchioles were evaluated by semi-logarithmic interpolation between the last bronchial generation and the first respiratory bronchioles. This procedure gave values of bronchial dead space as a function of age that are consistent with physiological data.

Figure 1 shows the calculated dependence on particle size of the conversion coefficient between exposure to Rn decay product potential α -energy

FIGURE 1. Calculated Dose Conversion Coefficients for Different Subjects Resting or Engaged in Light Activity (neglecting filtration of particles smaller than 10 nm diameter by the nose or mouth)

[expressed in the unit working level month (WLM)]^(a) and the mean dose received by secretory cells throughout the bronchi and bronchioles. Filtration of ultrafine particles by the nose or mouth, which is significant for particles less than about 5 nm diameter, is not considered in this figure. The model of mucous clearance and partial uptake of Rn decay products by epithelial tissue that was described by James (1988) was used for this calculation. However, the assumed clearance behavior was found to have a much smaller effect on estimates of dose than environmental variability in Rn decay product aerosol size. It is seen that the calculated dose conversion coefficient does not differ substantially between male and female adults, nor with subject age. Also, the dose conversion coefficient was found to be relatively insensitive to the level of physical activity. The minute volume is higher by a factor of three for adults and for the 10-year-old child between rest and light exercise; yet, over the particle size range 10 nm to 0.3 μ m, the dose for exposure to a given concentration of Rn decay products is higher by only about 60%. However, for unattached Rn decay products (in the size range 0.6 to 3 nm) and for

(a) Working level (WL) is defined as any combination of the short-lived decay products in 1 L of air that will result in the ultimate emission of 1.3×10^5 MeV of potential α -energy. WLM is an exposure equivalent to 170 h at a 1-WL concentration.

large particle sizes that may be relevant to exposure in mine environments, the dose conversion coefficient was found to approach proportionality with the subject's breathing rate (minute volume).

The Effect of Nasal and Oral Filtration

Cheng et al. (1989) have studied the deposition of unattached ^{212}Pb in several hollow casts of both the nasal and oral passages of adult male subjects. For a measured particle size of 1.8 nm (diffusion coefficient of $0.02 \text{ cm}^2 \text{ s}^{-1}$), corresponding to aged "molecular clusters," they found that the fraction of inspired activity able to traverse the nasal passages varied between 15% and 35%. On comparison with earlier measurements for particles larger than 5 nm diameter, Cheng et al. (1989) concluded that the penetration efficiency of the human nose is represented in terms of flow rate (in L/min) and particle diffusion coefficient by the empirical expression:

$$P = \exp(-13.2 Q^{-1/8} D^{1/2}) \quad (2)$$

The penetration efficiency predicted by Equation (2) for the smaller particle size of unattached ^{218}Po and ^{214}Pb found typically in indoor air (1 nm diameter, diffusion coefficient $0.04 \text{ cm}^2 \text{ s}^{-1}$) is approximately 15%. Cheng et al. (1989) found essentially the same penetration efficiency for the oral passageway. Strong and Swift^(a) also found similar penetration efficiencies for nasal and oral casts, using unattached ^{218}Po . However, their data implied a penetration efficiency of approximately 30% for 1-nm-diameter particles, i.e., twice that found by Cheng et al. (1989).

Figure 2 compares the nasal deposition efficiency predicted by Equation (2) with the experimental measurements in volunteer subjects made by George and Breslin (1969). The human data are consistent with the slight dependence of deposition on $Q^{-1/8}$ found by Cheng et al. (1989). The diffusion coefficient for ^{218}Po implied by these data and by Equation (2) is $0.0146 \text{ cm}^2 \text{ s}^{-1}$. Although the diffusion coefficient under the actual experimental conditions was not measured, this value is substantially lower than the expected range 0.03 to $0.04 \text{ cm}^2 \text{ s}^{-1}$. However, the lower deposition efficiency measured in casts by Strong and Swift

FIGURE 2. Nasal Deposition of Unattached ^{218}Po Measured in Three Subjects at Various Flow Rates, Compared with Values Predicted by Cheng et al. (1989) for Particle Diffusion Coefficients of $0.0146 \text{ cm}^2 \text{ s}^{-1}$ and $0.04 \text{ cm}^2 \text{ s}^{-1}$.

appears to be consistent with the experimental human data. Clearly, interpreting nasal and oral deposition measured in hollow casts requires further study.

To resolve the current uncertainty of about a factor of two in nasal penetration efficiency for unattached Rn decay products, it would be worthwhile pursuing further experimental measurements with human subjects. Also, it is necessary to confirm in vivo that the filtration efficiencies of the nose and mouth are indeed equal. Preliminary measurements of the filtration of unattached ^{218}Po in nasal casts from an infant and a child indicate that airway dimensions have a small effect on nasal deposition at the equivalent physiological flow rates (Strong and Swift).

Reference Dose Conversion Coefficients

Figure 3 shows the average dose to secretory cell nuclei throughout the bronchi and bronchioles from exposure to 1-WLM potential α -energy calculated for an adult male as functions of the activity median diffusion diameter (AMDD) of the Rn decay product aerosol and for various levels of physical activity. PNL has assumed that the deposition efficiency of the nose or mouth is represented by Equation (2). Higher penetration (by about a factor of two at 1 nm diameter) would increase the dose conversion coefficients for unattached Rn decay products to give relatively flat maximum values. It was found that the assumed clearance behavior of Rn decay products has a small effect on the dose

(a) Personal communication, 1989, National Radiological Protection Board, Chilton, United Kingdom.

FIGURE 3. Dose Conversion Coefficients Calculated for the Adult Male at Various Levels of Physical Activity, Assuming the Nasal Filtration Efficiency for Unattached Rn Decay Products Reported by Cheng et al. (1989)

to secretory cell nuclei averaged throughout the bronchial tree.

The distribution of doses to secretory cell nuclei in each bronchial and bronchiolar generation, calculated for exposure of a resting adult male to a "typical" indoor Rn decay product aerosol, is shown in Figure 4. It is assumed that the unattached fraction of potential α -energy is typically 10%, with a characteristic particle size of 1 nm. The attached aerosol is assumed to have an AMDD of $0.15 \mu\text{m}$, which is taken to grow to $0.3\text{-}\mu\text{m}$ AMDD in the humid air of the respiratory tract. It can be seen that unattached decay

FIGURE 4. Dose Conversion Coefficients Calculated for Secretory Cell Nuclei in Each Bronchial and Bronchiolar Airway Generation for a Resting Adult Male

products contribute most of the dose to cells in the bronchi, whereas the attached fraction contributes most of the dose to the bronchioles. The net effect is a relatively constant dose for all airway generations.

The revised dosimetry model is being used to compare doses to basal and secretory cell nuclei in the bronchi (and to secretory cell nuclei in the bronchioles) in subjects exposed to the range of Rn and Tn decay product aerosol conditions found indoors, with doses calculated for underground miners. The model is also being extended to evaluate doses to target cells in the bronchi and bronchioles of the laboratory rat, on the basis of the morphometric data reported by Mercer et al. (1989).

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Measurement
Science

Capillary Electrophoresis-Mass Spectrometry

The analysis of environmental, hazardous mixed waste or biological mixtures is best addressed by combined separation-mass spectrometry techniques. The development of these improved analytical methods rests upon the speed, selectivity, and efficiency of the separation combined with the sensitivity and flexibility of mass spectrometric analysis methods. This program is developing methods that are widely applicable to nonvolatile or highly polar compounds, interactable with more conventional methods such as gas chromatography-mass spectrometry. Currently, new methods based upon capillary electrophoresis-mass spectrometry (CE-MS) are being investigated. The goal of this research is to develop ultrasensitive CE-MS methods applicable at the attomole level for environmental and health-related problems.

Development of Capillary Electrophoresis-Mass Spectrometry

R. D. Smith, H. R. Udseth, and J. A. Loo

The history of analytical advances in MS has highlighted the special importance of the combination of separation methods having high selectivity and resolving power in conjunction with the high sensitivity and specificity of mass spectrometric detection. "Real-world" samples are invariably mixtures, and often very complex mixtures. Any useful analytical method must accommodate contributions from the sample matrix, interfering substances, etc. The dynamic combination of CE, a separation method of high efficiency and flexibility, with electrospray ionization (ESI)-MS is thus particularly advantageous.

Electrophoresis forms a family of related techniques, including polyacrylamide gel electrophoresis, isotachopheresis, isoelectric focusing, gel electrofocusing, and two-dimensional electrophoresis, for very complex mixtures (i.e., extraordinarily high peak capacity). These methods are of fundamental importance in biochemical analysis where different techniques are applied according to the type of sample and the information required. Electrophoresis can be conducted in narrow tubes [10- to 250- μ m inside diameter (ID)] with important advantages in efficiency and speed of analysis, and unique benefits when the manipulation of very small samples is mandatory. A suite of operational modes that are highly complementary to conventional electrophoretic methods are available in the capillary format including capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and capillary isotachopheresis (CITP).

The on-line combination of CZE with ESI-MS (Olivares et al. 1987; Smith et al. 1988b) was based upon the recognition that both ends of the CZE capillary did not have to be immersed in buffer reservoirs, as conventionally practiced. The capillary terminus was adapted as an ESI source that accepts the low (from 0 to 1 μ L/min) electroosmotic flow and allows ion production at atmospheric pressure from the electrostatically induced nebulization process. In earlier versions of the CZE/ESI interface (Olivares et al. 1987; Smith et al. 1988b), the electrospray dispersion and ionization were accomplished from an electrodeposited metal contact established at the end of the CZE capillary. Subsequently, a liquid sheath-electrode interface was developed from which the solvent composition and flow rate of the electrosprayed liquid could be controlled independent of the CZE buffer (which is desirable since high-percentage aqueous and high-ionic-strength buffers that are useful in CZE are not well tolerated by ESI) (Smith et al. 1988a). The electrical contact was established through the conductive sheath, no additional mixing volumes (<10 nL) were produced, no significant positive or negative hydrostatic pressure was produced at the outlet of the electrophoretic capillary (i.e., no induced flow) at normal flow rates of the sheath liquid (1 to 3 μ L/min), and analyte contact with metal surfaces was avoided.

The interface provides greatly improved performance and is adaptable to other forms of CE. Because CE relies on analyte charge in solution and the ESI process appears to function most effectively for ionic species, the CE/ESI-MS combination is highly complementary. It has been reported that the analysis by CZE/MS of a mixture of quaternary ammonium compounds (Olivares

et al. 1987) obtains over 600,000 plates, two orders of magnitude better than obtainable by liquid chromatography (LC) in similar time. In this case, sample sizes were ~ 300 femtomoles; however, detection limits of ≤ 10 attomoles are obtainable using single ion detection. Thus, the CZE/MS approach offers previously unobtainable separation efficiencies (for the combination with MS) as well as detection limits that can greatly surpass existing methods.

As initially demonstrated by Lauer and McManigill (1986), high-efficiency CZE separations of protein mixtures can be obtained with unmodified fused silica capillaries by using buffer systems at a pH above the isoelectric points of the analytes, and thus allowing the peptides and proteins to be negatively charged. The detection of anions in this separation mode is handicapped by reduced production or outright absence of multiply charged negative ions for peptides and higher molecular weight (M_r) proteins by ESI from such high-pH solutions. However, multiply charged positive ions can still be produced and detected from peptide and protein samples that are negatively charged in solution by the addition of an aqueous acetic acid solution to the methanol sheath electrode. This charge-state inversion is illustrated in Figure 1 for the pH 8.3 separation obtained using a pH 8.25, TRIS-HCl buffer and a 100-cm $\times$ 50- μ m untreated fused silica capillary of sperm whale skeletal muscle myoglobin (M_r 17,199; pI 8.1) and horse heart myoglobin (M_r 16,950; pI 7.4) with detection by ESI-MS in positive ion mode. The ion signal for each analyte is distributed among its several charge states, three of which are shown.

In isotachopheresis, all analytes separate into bands in order of decreasing electrophoretic mobility, and upon complete development, progress through the separating medium at the same velocity. Each solute band stacks in decreasing order of mobility and all bands are moving at the same velocity. The steady-state concentration of the analyte anion is determined by the lead anion concentration and the relative mobilities of the solute, lead anion, and common cation. Accordingly, if the analyte is much more dilute than the lead anion concentration (which is the case in many analytical separations), the analyte will be *concentrated* as it separates into its own band. Thus, capillary isotachopheresis offers the potential for higher sample loading (and increased

molar sensitivity), high-resolution separations, and actual concentration (in many cases) of separated samples bands.

CITP is an attractive complement to CZE and is ideally suited for combination with MS. The feasibility of the isotachophoretic separation of quaternary phosphonium and ammonium salts, amino acids, catecholamines (Udseth et al. 1989), and reference peptides (Smith et al. 1989b) with detection by ESI has been demonstrated. Detection limits of approximately 10^{-11} M have been demonstrated for quaternary phosphonium salts and substantial improvements appear feasible (Udseth et al. 1989). Sample sizes that can be addressed by CITP are much greater (>100 -fold) than CZE. CITP results in concentration of analyte bands, which is in contrast to the inherent dilution obtained in CZE. Samples eluting in CITP are ideally flat-topped bands (the length of the analyte band provides information regarding analyte concentration), well suited to MS as the scan speed of the mass spectrometer need not be challenged by the dynamic nature of "sharp" peaks. Most importantly, however, is that CITP provides a relatively pure analyte band to the ESI source, without the large concentration of a supporting electrolyte that is demanded by CZE. Thus, CITP/MS has the potential of allowing much greater sensitivities (and analyte ion currents) than may be possible by CZE/MS.

CITP/MS is also complementary to CZE/MS in many ways. The CZE column is easily overloaded, whereas CITP tolerates very high loading. Thus, larger injection volumes (0.1 to 1.0 μ L) of dilute sample solutions can be analyzed. Electromigration injection allows effective sample volumes to be much larger still due to enrichment during migration into the capillary from low-ionic-strength samples. CITP is well suited to low concentration samples where the amount of solution is relatively large, whereas CZE is ideal for the analysis of minute quantities of solution.

Figure 2 shows isotachopherograms for the CITP/ESI-MS analysis of a mixture of peptides derived from the digestion of glucagon (M_r 3483) with trypsin. The times shown on the axis are marked from the beginning of the separation. Separation development in CITP may be accomplished off-line and, as shown here, the actual time for CITP/MS analysis is determined by the

FIGURE 1. CZE/ESI-MS Selected Ion Monitoring Separation of Sperm Whale and Horse Myoglobins (Smith et al. in press)

application of a hydrostatic head to the separation capillary with elution of the focused bands (at 27 min). The selected ion isotachopherograms are shown in Figure 2 for the tryptic products arginine (Arg^+ , m/z 175), Trp^{2+} (m/z 676) and T^{2+} (m/z 653). Also detected are additional peptide fragments that arise from the action of contaminating chymotrypsin on the predicted tryptic fragments (T4-478^{2+} and (T4-1049)^+ and on intact glucagon (m/z 546 and 366).

The combination of CE, including CZE and CITP, with ESI-MS provides a powerful analytical tool for the analysis of biologically related compounds.

The ESI process presently provides detection limits in the attomole-to-femtomole range (Barinaga et al. 1989; Loo et al. 1988a, 1988b, and 1989). The multiple-charging phenomena observed in the ESI mass spectra of large biomolecules also have two important advantageous effects. Firstly, the mass range for analysis with conventional quadrupole mass spectrometers is extended by a factor equal to the extent of charging. This provides mass spectrometric resolution and precision of mass measurement equivalent or superior to mass spectrometric methods dedicated to the measurements at high m/z . Secondly, the efficiency of collisionally induced dissociation

FIGURE 2. Electrospray Total Ion Current Isotachopherogram (upper) and Corresponding Ultraviolet Absorbance (210 and 280 nm) Traces (lower) for the CITP/MS Analysis of a Tryptic Digest of Glucagon. 2-m x 100- μ m column, leading buffer 0.01 M NH_4OAc , pH 4.9; sample concentration 0.0001 M; 25% column volume loading; trailing buffer 0.01-M acetic acid, pH 3.3.

(for MS/MS) is markedly enhanced (Smith et al. 1989a).^(a) This provides for an important flexibility and advantage in the qualitative investigation of analytes above the range of mass ordinarily accessible for singly charged analyte parent ions.

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Lasers in Environmental Research

This program has shown in previous years that double-resonance ionization mass spectrometry (DRIMS) with high-resolution continuous wave (cw) lasers can provide analytical methodology for the measurement of rare isotopes at abundances of less than 1 part in 10^{12} with respect to other isotopes of the same element (Bushaw 1989). Experimental efforts began this year that apply high-resolution multiphoton spectroscopic techniques to the investigation of molecular species. In addition to exploring analytical applications, this work will yield a basic understanding of both the detailed structure of small molecules and their complex decay dynamics through processes such as autoionization and dissociation. Initial studies on nitric oxide (NO), an important atmospheric pollutant generated in energy production, resulted in the first measurement of hyperfine interactions in the $v = 1$ vibrational level of the first excited state; demonstration of the generation of full quantum specified excited states; and demonstration, for the first time, of a cw-cw new double-resonance spectroscopic technique for the measurement of predissociation rates in higher lying Rydberg states of the molecule. The higher resolution available under these excitation conditions will yield unprecedented information on the structural and dynamical properties of excited electronic states.

Ultrahigh Resolution Spectroscopic Studies of Nitric Oxide

B. A. Bushaw, R. J. Miller, and W. L. Glab

The molecule NO is of particular interest because it plays a significant role in the chemistry and physics of the upper atmosphere: it is directly involved in the ozone cycle and is a major industrial pollutant. A detailed understanding of its electronic structure and excited state dynamics will contribute to the understanding and modeling of these processes.

Fine and Hyperfine Structure in the $(3s\sigma)A^2\Sigma^+(v = 1, N = 3)$ State of NO

Fine and hyperfine structure splitting in the spectra of molecules are the result of fundamental physical interactions within the given molecule. Parameters characterizing magnetic hyperfine interactions yield detailed structural information about the orbital and spin distributions of unpaired electrons; the nuclear electric quadrupole coupling describes the electrostatic interactions of all the charged particles within the molecule. These interactions are very important for a complete description of the structure of a molecule, and, for the ground state, these interactions can be readily observed in microwave rotational spectroscopy. However, for excited states studied by optical spectroscopy, the relative magnitude of these effects is very small and difficult to observe.

Despite the pioneering work of Timmermann and Wallenstein (1981), the consensus remains that only pulsed lasers can provide the field intensities required for multiphoton excitations of molecular species without resonant intermediate states. However, the resolution achievable with pulsed lasers can never exceed the Fourier transform limit; frequently this resolution is insufficient for measurements of the structure and decay dynamics of the excited states of interest (Biernacki et al. 1988). The enhanced resolution achievable with cw excitation removes this restriction, allowing the measurement of small fine and hyperfine splitting, as well as the determination of line shapes from which slow predissociation or autoionization rates may be extracted. Because of these considerations, we have applied cw single-mode dye lasers in Doppler-free, two-photon induced fluorescence spectroscopy for the study of the fine and hyperfine interactions in the first excited electronic state of NO (Miller et al. 1989). These experiments involved counterpropagating the focused laser beam through a static cell containing (typically) 50 millitorr of NO, thereby inducing the two-photon excitation to the A state. The resulting A state population was monitored by observing ensuing one-photon fluorescence decay back to the ground state. Figure 1 shows the observed signal in the vicinity of the P bandhead and illustrates how this technique can fully resolve all the rotational components, even in highly congested regions of the spectrum. Figure 2 gives the

FIGURE 1. High-Resolution, Doppler-Free, Two-Photon Excitation Spectrum of the $A(v=1)-X(v=0)$ Transition in the Vicinity of the P Bandhead. The small, broad pedestals at the base of each peak are the Doppler-broadened transitions due to absorption of both photons from the same direction.

FIGURE 2. Hyperfine Structure in the $R_{11}(J' = 2.5)$ Rotational Line. Points are the experimental data and the solid line is the theoretical spectrum calculated from Clebsch-Gordon coefficients (intensities) and the coupling constants described in the text. Labels are the hyperfine quantum number F for the upper and lower states.

spectrum of a single rotational branch (R_{11} , $J' = 2.5$) at higher resolution (500 times that in Figure 1) and shows that the hyperfine components of the individual rotational branches can also be clearly resolved. The couplings that produce the splitting can be characterized by the zero-field spin Hamiltonian:

$$H = \gamma(\mathbf{N} \cdot \mathbf{S}) + b(\mathbf{I} \cdot \mathbf{S}) + cI_zS_z + \frac{eQq[3I_z^2 - I(I+1)]}{4I(I-1)} \quad (1)$$

where $\mathbf{N}$, $\mathbf{S}$, and $\mathbf{I}$ are, respectively, the nuclear rotation, electron spin, and nuclear spin angular momenta vectors; I is the scalar magnitude of $\mathbf{I}$; and I_z and S_z are projections onto the internuclear axis. The fine structure parameter γ and the hyperfine structure parameters b , c , and eQq have been extracted from the experimental splitting shown in Figure 2, as well as those measured in five other rotational lines, by diagonalization of the matrix elements derived from Equation (1). The resulting values are:

$$\begin{aligned} \gamma &= -80.10 \pm 0.04 \text{ MHz} \\ b &= 40.9 \pm 0.2 \text{ MHz} \\ c &= 1.5 \pm 4.5 \text{ MHz} \\ eQq &= -7.2 \pm 0.4 \text{ MHz} \end{aligned}$$

Also, from analyses of the absolute signal intensities, the two-photon excitation cross section (for the R_{11} rotational line) was determined to be $1 \times 10^{-44} \text{ cm}^4 \text{ s}$.

Term Energies and Predissociation Rates of Individual Rovibronic Levels in the K Rydberg State: Velocity-Selected, Double-Resonance Fluorescence Dip Spectroscopy

cw-cw double-resonance spectroscopy was implemented to characterize higher lying Rydberg states in NO. The first excitation step is similar to the two-photon excitation to the A state described above; however, the laser excitation beam was not counterpropagated. Thus, both photons must be absorbed from the same direction and hence have a Doppler dependence: only specific velocity groups within the thermal distribution are in resonance with the laser radiation. Molecules prepared in the A state have a well-defined velocity along the direction of laser propagation (actually there is a set of distinct velocity groups corresponding to each of the hyperfine levels). A second laser, collinear with the first, can then be used to promote the molecules from the A state to some higher level in a Doppler-free manner. In the initial experiments, promotion of the NO molecules to the predissociative $(4\rho\pi)K^2\Pi(v=2)$ Rydberg state by the second laser was detected as a reduction in the one-photon fluorescence intensity

from the A state (hence the name fluorescence dip). However, the technique is also suitable for upper states that can be detected either by direct fluorescence or by ionization. Figure 3 shows a typical fluorescence dip spectrum for the Q_{11} ($J^* = 3.5$ in A state) rotational line. The large width of the line (570 MHz) is caused by lifetime broadening due to rapid predissociation in the K state, with the lifetime given by $\tau_p = 1/2\pi\Gamma$, where Γ is the measured Lorentzian linewidth (full width at half maximum). In addition to determining the lifetimes of individual rovibronic levels, very accurate and precise absolute energies can be assigned because of the high resolution of the lasers and the ability to measure their frequencies with systems developed under the earlier atomic spectroscopy aspects of this program. Thus far, the energies and lifetimes (ranging from 190 to 410 picoseconds) have been determined for six

FIGURE 3. Velocity-Selected, Double-Resonance Fluorescence Dip Spectrum of the $J = 3.5$, $\Omega = 0.5$ Rotational Level of the K Rydberg State. The large width is due to the fast predissociation; for comparison, the inset shows the spectrum of a nondissociating level [tentatively assigned to the $(3d\delta)F^2\Delta(v=3)$ Rydberg state] in which structure due to the hyperfine splitting (see Figure 2) in the intermediate A state is observed.

different rovibronic levels in the K state. These results are summarized in Table 1. This work is continuing, examining a larger range of rotation levels to evaluate the interactions with nearby perturbing valence states.

TABLE 1. Term Energies and Lifetimes of Rotational Levels of $K^2\Pi(v=2)^{14}N^{16}O$

Level, J, Ω	Energy, vacuum cm^{-1} (a)	Lifetime, ps (b)
2.5, 0.5	69032.078	410 (6)
3.5, 0.5	69043.229	278 (6)
2.5, 1.5	69054.923	192 (5)
4.5, 0.5	69057.358	311 (10)
3.5, 1.5	69065.106	189 (7)
4.5, 1.5	69079.715	255 (11)

(a) Cumulative absolute accuracy is 0.005 cm^{-1} .

(b) Values in parentheses are uncertainties derived from 4 to 7 repetitive measurements.

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Radiological
and Chemical
Physics

Radiation Physics

The initial spatial distribution of energy deposition and the interaction channels that lead to this deposition pattern play a significant role in the subsequent evolution of chemical and biological damage. Radiochemical and radiobiological models of radiation action rely on an accurate description of the fundamental processes of energy deposition and energy transport to provide a link between energy deposition and chemical and biological damage. For radiation of high linear energy transfer (LET), such models require a detailed description of the physical processes that occur along charged particle tracks. Studies in the Radiation Physics Project focus on investigation of the physical processes of ionization by charged particles that contribute to the structure of charged particle tracks, on the fate of the atom or molecule that undergoes an ionizing event, and incorporation of these data into Monte Carlo models of charged particle track structure.

During the past year, the experimental studies of energy deposition by fast ion impact have concentrated on three primary tasks: 1) the study of interaction channels leading to energy deposition in collisions of light ions (H^+ , H^0 , He^+ , etc.) with atoms and molecules, 2) the study of electron emission in fast heavy ion-atom collisions, and 3) development of the instrumentation to investigate electron production in the condensed phase targets. Studies in the simulation of charged particle track structure have focused on the development of Monte Carlo techniques to investigate the distribution of energy deposited in small tissue-equivalent volumes near the path of high-energy heavy ions.

Mechanisms for Energy Loss in Collisions of Charged Particles with Atomic and Molecular Targets

R. D. DuBois

As a charged particle slows down in passing through matter, its primary means of energy loss is through ionization of the atomic and molecular constituents of the medium. Electrons produced by these ionizing collisions are the principal means of energy transport and the source of the structure of the charged particle track. Measurements of the spectra of electrons emitted in ionizing collisions provide basic information needed to describe the structure of these tracks. However, these data do not provide information on the electronic states of the residual targets, nor do they provide information on the potential for the simultaneous emission of more than one electron. Both of these processes may have important consequences for assessment of the subsequent evolution of chemical and biological damage.

For several years, the role of multiple ionizations in the production of electrons by ionization and charge transfer in collisions of bare ions with atomic targets has been investigated. In these measurements, total cross sections for the production of specific target ion states were

measured. From those data, information concerning the total ionization and charge-transfer cross sections was obtained. In addition, measurements were made in which the residual target charge state was determined in coincidence with the post-collision charge state of the ion. These measurements provide information regarding the number of free electrons produced in a single collision and the relative importance of the various ionization channels leading to the production of free electrons. Such data can be useful in guiding the theoretical models of energy deposition used in track structure calculations, particularly near the end of an ion's path where the charge state of the ion may change as a result of electron transfer collisions. During the past year, these studies have been extended to the investigation of incident particles that carry bound electrons and to the study of dissociative ionization of molecular targets.

PNL's initial study of ionization channels for structured ions focused on multiple ionization of atomic targets by He^+ ions (DuBois 1989). This study for He, Ne, Ar, and Kr targets indicated that multiple ionization of the target plays a significant role in the production of free electrons. The data in Figure 1 illustrate the fraction of the free electrons that are produced as a result of collisions involving multiple ionization of the target atom; the

FIGURE 1. The Fraction of Free Electrons Resulting from Multiple Ionization of Helium Atoms by He^+ Ion Impact

results shown in Figure 2 illustrate that electron capture and loss channels contribute significantly to the electron production process at low and high energies, respectively. These studies also showed that ionization of the projectile will generally result in the simultaneous ionization of the target. This may have biological consequences since it results in the release of two time and spatially correlated electrons.

In addition to the study of electron production by He^+ , a study of single and double ionization of He by H atom impact was also completed. This study of the influence of bound projectile electrons (DuBois and Köver 1989) was made in collaboration with A. Köver Institute of Nuclear Research, Debrecen, Hungary. The $\text{H}^0\text{-He}$ collision system was chosen as the simplest system that was available to provide a basic test of collision theory. The results of the measurement indicated that although theory does an excellent job in describing total ionization of the target and projectile, it tends to underestimate ionization of the target or projectile, independently, and to overestimate the probability of simultaneous target and projectile ionization.

The study of atomic targets has been productive in providing information on the general trends that may be expected regarding the relative importance of different ionization channels. These data also provide a sensitive test of collision theory. However, how these systematics may be altered by the presence of additional degrees of freedom

FIGURE 2. Interaction Mechanisms Leading to the Production of Free Electrons in Collisions of He^+ Ions with Neon Atoms

that are available in molecular and condensed phase targets is of radiological importance. To begin to address this problem, studies of the dissociative ionization of simple molecules have been initiated. The same basic experimental technique is used in the measurements, namely target ions are extracted and detected in coincidence with the postcollision charge state analyzed projectile. The only experimental change required for molecular targets is that a higher extraction field is needed because molecular dissociation fragments are produced with much higher energies than comparable ion states produced from atomic targets. Unfortunately, this larger extraction field, 2000 V/cm, limits the minimum impact energy that can be used to about 200 keV owing to deflection and thereby loss of the ion beam in the extraction region of the target.

Initial measurements were carried out on two homonuclear diatomic molecules, N_2 and O_2 . Absolute cross sections for the various charged ionization/dissociation fragments were measured as a function of impact energy (200 to 2000 keV), collision type (ionization, electron capture, electron loss), and projectile ion species (H^+ , H^0 , He^{2+} , He^+ , C^{q+}).

Of primary interest in these studies is the determination of how the population distributions of dissociation fragments compare with the distributions measured for electron or photon impact. A comparison of the fractions of the various molecular and atomic fragments of N_2 are shown in Figure 3 as a function of impact velocity for various projectiles colliding with molecular nitrogen. Note that these data are for the pure ionization channel only; contributions from collisions involving electron capture or loss by the projectile are not included. From the preliminary data shown in Figure 3, some general conclusions can be drawn: 1) for the larger impact energies, the H^+ , H^0 , and e^- data show similar fragmentation patterns; 2) at smaller impact energies, dissociation channels become more important relative to ionization of the molecule as a whole; and 3) the dissociation fractions are generally independent of the projectile ion species, except possibly in the case of the production of multiply charged fragment ions, which seem to be more readily produced by the heavier ions. On the other hand, some of the dissociation fragments are found to depend

FIGURE 3. The Fraction of Collisions Involving Production of Various Charged Fragments in the Dissociative Ionization of N_2

strongly on the type of collision and impact velocity. Measurements of three of the charged products from ionization of N_2 by He^+ are shown in Figure 4 as they result from direct ionization, projectile electron loss plus target ionization, and projectile electron capture plus target ionization. Note the large difference in the yields depending on the fragment, the ion velocity, and the interaction type. These data indicate the complexity and scope of the database needed to incorporate molecular dissociation into the description of energy deposition by charged particles.

FIGURE 4. The Fraction of Collisions Leading to Production of Various Target Charge States from Direct Ionization, Electron Loss, and Electron Capture in Collisions of He^+ with N_2

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Electron Emission in Fast Heavy Ion Collisions

R. D. DuBois

Extensive data exist concerning the differential cross sections for electron emission in collisions between bare charged particles and atomic and molecular targets. Considerably less is known regarding this process for ions that carry bound electrons. In order to provide information to partially fill this void, PNL has entered into a collaborative study of electron emission with research groups at the Lawrence Berkeley and Lawrence Livermore Laboratories. The initial studies have focused on 6 MeV/amu U^{38+} and Th^{38+} ions colliding with Ar targets. The importance of these measurements is that they provide data on structured ions at energies sufficiently high that the high-energy Born theory can be expected to be reliable. The high projectile charge was selected to investigate charge-state scaling properties of the cross sections. It is anticipated that these data can provide a benchmark test of theoretical techniques that can then be extended to lower energies.

Double differential cross sections for electron emission from 6 MeV/amu U^{38+} collisions with Ar are shown in Figure 1 along with Born calculations. These data indicate that scaled Born calculations do a reasonably good job in describing the experimental results, although systematic deviations were found for forward and backward emission angles. These deviations are attributed to electron emission that occurs in the combined coulomb fields of the projectile and the target that tends to shift the electron distributions to the forward direction.

Differential Cross Sections for Electron Emission in Heavy Ion Collisions

L. H. Toburen

Electron emission from low- and intermediate-velocity ions colliding with atomic and molecular targets has been the subject of study at PNL for a number of years. Recent emphasis has been on the study of doubly differential electron emission cross sections for C and O ions in the energy range from 0.8 to 4.2 MeV. For these ion velocities, there has been no reliable theory with which to compare measured results that can provide

FIGURE 1. Doubly Differential Cross Sections for Electron Emission in Collisions of 6 MeV/amu U^{38+} with Argon

guidance as to the different ionization mechanisms responsible for the observed spectral features. However, recent success in the application of the Classical Trajectory Monte Carlo (CTMC) technique to electron emission in collisions of somewhat higher energies (Schmidt et al. 1989) has encouraged PNL to investigate this technique for ions in this energy range. The possibilities of such calculations were discussed with Dr. Carlos Reinhold of the University of Missouri, Rolla, who has made such calculations for other systems. Dr. Reinhold has now initiated CTMC calculations for the collision C^+ on He, for which PNL has experimental data. His preliminary results for the single differential cross sections have been highly

encouraging. A comparison of Dr. Reinhold's calculations to PNL's measurements for ionization of He by 200-keV/amu C^+ ions is shown in Figure 1. Overall there tends to be excellent agreement between the calculated and measured cross sections; this is to be compared to calculations based on binary encounter theory that give only order-of-magnitude estimates at the very best. From comparison of the measured and calculated spectra, it would appear that the theory may overestimate the contribution to the spectra from ionization of the projectile. Calculations are now being extended to the determination of doubly differential cross sections to further explore the contributions of target and projectile electrons to the total spectra of ejected electrons. Additional measurements are also planned to check the reproducibility and the accuracy of the experimental values.

FIGURE 1. Single Differential Cross Sections for Electron Emission from 200-MeV/amu C^+ Collisions with He. The theoretical results are a CTMC calculation provided by Dr. Carlos Reinhold of the University of Missouri, Rolla.

Reference

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Electron Emission from Condensed Targets

R. D. DuBois

The study of electron emission has traditionally focused on the measurement of electrons ejected from atomic and molecular targets by proton and other light ions. Data from such single-collision systems provide basic information on the ionization process and insight into the effects of target structure on collision cross sections. However, effects of condensed phase on these cross sections must be derived either totally from theory or from optical data coupled with theory. In order to provide data from condensed phase targets to begin to test our models of condensed phase energy transport, PNL has been developing a new experimental system to study electron emission from solid targets. During the past year, considerable progress has been made in assembling this system. The apparatus, shown schematically in Figure 1, consists of an ultrahigh vacuum chamber

FIGURE 1. Schematic of Chamber Developed for the Study of Electron Emission from Condensed Targets

containing a time-of-flight electron spectrometer capable of measurements as a function of the angle θ with respect to the ion beam. The time-of-flight spectrometer is mounted on an xyz positioner for accurate alignment of the spectrometer to the beam and target. The target consists of a foil holder assembly capable of holding several different foils that can be remotely changed and centered on the beam. In addition, gas-phase targets can be introduced by a hypodermic needle assembly positioned near the foil target holder. Thus gaseous and foil targets can be directly compared with a minimum of experimental modification. A turbomolecular pump will be used for gas target measurements where a large gas load is required. For foil measurements, the turbomolecular pump can be valved off and ultrahigh vacuum conditions obtained by an ion pump.

Modeling the Stochastic Track Structure of High-LET Radiations

W. E. Wilson

The analytic model for ion crossers (Wilson et al. 1988) was updated. Interactive graphical analysis software provided the opportunity to reanalyze computed Monte Carlo results from 0.3- to 20-MeV protons crossing simulated sites of 2 to 1000 nm diameter. A more thorough analysis facilitated by computer-aided data visualization has allowed a simplified crosser model. Some cases were recomputed in order to evaluate the implications of a discovered error in previous simulations and to take advantage of recent improvements made in the algorithms for track structure. The qualitative features of the model still apply. The frequency distributions are well described by the log normal function, however the first and second moments of the distributions are increased by roughly 10% overall.

The simulation and analysis of touchers, ions that pass outside a site but deposit energy inside through delta rays, was resumed. The basic problem is to develop an analytic model for the

stochastics of the penumbra around an ion track. This problem was studied previously, but with inefficient scoring procedures. The radial dependence of the total hit frequency was determined, but the frequency distributions for energy imparted could not be computed with adequate statistical accuracy. The scoring procedures used for the crosser problem were simply too inefficient for touchers.

A more efficient scoring scheme specifically for toucher geometry has been developed and tested. Also, a numerical method for rapidly sorting real numbers into bins was invented. This process is the computational equivalent of sorting electronic pulse heights into spectra by a pulse height analyzer. A great advantage of this numerical method, in addition to speed, is that a wide dynamic range can be accommodated with only a few computer memory cells because the scaling is done on a logarithmic (base two) scale. A dynamic range of over 1 million can be covered with just 80 memory locations.

Preliminary frequency distributions in energy imparted for 1-MeV proton touchers depositing energy in sites of 2 to 100 nm diameter have been computed. These early results appear to confirm the experimental observation (Toburen and Metting 1989) that for a given site diameter, the shape of the energy deposition distributions is independent of radial distance, beyond some minimum distance. Computations for additional ion energies and site sizes are in progress.

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Radiation Dosimetry

The primary goal of the Radiation Dosimetry task is to understand the connections between physical events produced by the interactions between ionizing radiations with matter and their biological consequences. Both the physical aspects of the problem and the response of cells to carefully controlled irradiation are studied. Fundamental physical data are used to develop a description of the interactions between ionizing radiation and biological materials; the results are characterized in ways that can be correlated with the biological effects. Special mathematical and experimental techniques are developed to explore the differences in the energy deposition characteristics of various radiations in an effort to understand the effects of these radiations. Experimental tests of specific aspects of track structure calculations are conducted to test the completeness of these models and the adequacy of the input data. Finally, comparisons are made with other methods of characterizing the energy deposition by charged particles, such as the homogenous track models, in order to help identify those characteristics that are relevant to the response on cells.

In order to determine which characteristics of energy deposition are relevant to the production of biological damage, various models of the response of cells are compared and tested. To identify the characteristics of the cellular response that depend on these physical properties of the irradiation, it is also necessary to characterize the biological processes which modify that damage. Models that are characteristic of specific biochemical mechanisms or groups of mechanisms are contrasted to determine whether they produce different consequences at the cellular level. Suitable experiments are designed to test differences that would be statistically significant at the precision of realistic experiments. In general, experiments using well-characterized cell lines are preferred, since more can be learned about specific mechanisms and their interactions by studying one system in detail, rather than checking one aspect of the response of many different systems.

Microdosimetry of Fast Heavy Ion Beams

N. F. Metting, L. H. Toburen, and L. A. Braby

During the past year, the analysis of data has continued on the distributions of specific energy deposited in nanometer-sized tissue-equivalent volumes that were measured as a function of the radial distance from the path of 5.9-MeV/amu U and 13.0- to 17.2-MeV/amu Ge beams. These data were obtained in collaboration with the research groups of Dr. G. Kraft, Gesellschaft für Schwerionenforschung (GSI), Darmstadt, and Dr. H. Schmidt-Böcking, University of Frankfurt, West Germany. Measurements were conducted at the UNILAC accelerator in Darmstadt. A preliminary analysis and a discussion of the experimental procedure have been presented in previous reports (Metting et al. 1987 and 1989; Toburen et al. 1989). This year, analysis of the U ion data was completed and the results presented at the Microdosimetry Symposium held in Rome in May 1989; a manuscript describing the results is in press as part of the symposium proceedings.

The mean specific energy deposited in simulated tissue volumes of 250 and 500 nm diameter is shown in Figure 1 as a function of the distance from the path of a 17.2-MeV/amu Ge beam. This is a typical example of the results obtained from the experiments. Note the rapid fall-off in mean specific energy for single events, $\bar{z}_b$, as the radial distance, b , increases from 0 to about 2 detector radii. The value of $\bar{z}_b$ becomes nearly constant as the distance increases further. This constant value of $\bar{z}_b$ for large b illustrates the stochastic nature of the energy deposition process. At the larger distances, the probability of energy deposition by the passing ion decreases, but the energy deposited by such an event (that deposited by a delta ray or absorption of a photon) is nearly independent of radial position. The differences in $\bar{z}_b$ for the two different site sizes reflects the energy/mass definition of z ; if values of the lineal energy, $\bar{y}_b$, are plotted instead of $\bar{z}_b$, the results for the two sites coincide. The probability of energy deposition per ion traversal, R_b , was also determined in these measurements. If the measured $\bar{z}_b$ are multiplied by R_b , the average energy deposited per ion thus obtained can be compared to calculations or

FIGURE 1. Mean Specific Energy ($\bar{z}_b$) Deposited in 500-nm ($\square$)- and 250-nm ($\circ$)-Diameter Simulated Tissue Volume as a Function of Radial Distance from the Path of a 17.2-MeV/amu Ge Beam. The solid symbols represent the product of the mean specific energy and the probability of an energy deposition event per ion track. The theoretical curves are from the work of Zhang et al. (1985), —; Chatterjee and Schaefer (1976) - - -; and Varma et al. (1978), - · -.

measurements of the radial distribution of dose, $D(b)$. The average dose obtained from our measurements, shown as the solid points in Figure 1, is in excellent agreement with calculations using the models of Zhang et al. (1985) and Chatterjee and Schaefer (1976). The measured values are, however, about a factor of two larger than the predictions of Varma's model (Varma et al. 1978). Also shown in Figure 1 is the penumbra radius, R_p , for 17.2-MeV/amu ions calculated using the model of Chatterjee and Schaefer (1976). There seems to be no evidence of a decrease in the measured $\bar{z}_b$ at the penumbra radius.

Data for energy deposited in three different simulated site sizes near the path of 5.9-MeV/amu U ions are shown in Figure 2. In this case, the plotted lineal energy, $\bar{y}_b$, is measured as a function of the radial distance for sites of 125, 250, and 500 nm diameter. As expected, the lineal energy deposited at distances greater than about 1 μm is independent of site size. The differences in the values at smaller distances reflect the finite size of the detector; a much greater amount of energy is deposited by the ion passing through the detector than is deposited by secondary electrons produced when the ion passes nearby. One can readily see the effect of the detector size on the

radial distribution of dose in Figure 3, where the measured mean specific energy is plotted as a function of radial distance for a 5.9-MeV/amu U ion passing through, or near, a 0.5-nm-diameter simulated tissue volume. The solid line in Figure 3 was calculated by simply multiplying the stopping power of the ion by the path length through the detector; a factor of 0.5 was necessary to

FIGURE 2. The Mean Lineal Energy ($\bar{y}_b$) Measured in 125-nm (+), 250-nm ($\square$), and 500-nm ($\circ$)-Diameter Simulated Tissue Volumes as a Function of the Radial Distance from a 5.9-MeV/amu U Beam

FIGURE 3. The Mean Specific Energy ($\bar{z}_b$) Deposited in a 500-nm-Diameter Simulated Tissue Volume. The solid line is derived from the product of path length of the ion through the detector and the ion stopping power; this product is multiplied by 0.5 for normalization to the measured values at $r = 0$.

normalize the calculated value to the measured dose. It is expected that the lineal energy estimate based on the chord length and stopping power would be larger than the measured value due to escape of secondary electrons from the detector volume; however, the factor of 2 difference is larger than expected and larger than that observed for the higher energy Ge beam. This difference may reflect intratrack charge recombination, but a clear explanation will require further analysis and, perhaps, additional data.

Measured values of $\bar{z}_b$ for the U beam are compared to model calculations for energy deposition in a 125-nm-diameter simulated tissue site in Figure 4. Again, as was observed for Ge ions, the measured energy deposition produced by an actual event in the detector at the larger distances from the ion path is greater than the calculated dose, whereas the average energy deposited per ion is in excellent agreement with the model calculations. The measured mean specific energy departs from the calculated average dose at a radius of about three times the simulated diameter for the site. This difference between the stochastic and average doses occurs at a somewhat larger radial

FIGURE 4. Comparison of the Measured Mean Specific Energy ($\bar{z}_b$) (o) and the Average Energy Per Ion ($\bar{z}_b \cdot R_p$) (□) Deposited in a 125-nm-Diameter Simulated Tissue Volume as a Function of Radial Distance from the Path of a 5.9-MeV/amu U Ion with Model Calculations. The calculations are from Zhang et al. (1985), —; Chatterjee and Schaefer (1976), - - -; and Varma et al. (1978), ——. R_p is the penumbra radius given by Chatterjee and Schaefer.

distance than observed in the Ge data and illustrates that there is a larger number of delta rays, per unit path length, produced by U ions of this velocity than by the higher energy Ge beam.

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Large Deletions and Mutation or Reproductive Death

T. L. Morgan, J. M. Nelson, and L. A. Braby

Although it has not been established beyond doubt, it appears that the radiation-induced reproductive death of cells results from the same or very similar mechanisms responsible for

producing mutations such as those that occur at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus, specifically, mechanisms that produce large deletions. One concern with the use of the HPRT mutation system for the analysis of deletion lengths is that the longest deletions will not be detected because they contain essential genes, and cells with those deletions will not survive to grow into clones. The inverse of this argument, of course, is that at least some lethal events are large deletions. That almost all lethal events, at least in G_1 cells, are deletions is evidenced from work in Revell's laboratory (Joshi et al. 1982), which shows an almost perfect correlation between reproductive death and the formation of micronuclei at the first mitosis after irradiation. Since the micronuclei hold DNA that has been cut out of normal chromosomes, this strongly suggests that the deletion of a significant amount of DNA is the lethal event.

Almost all radiation-induced mutations are the result of deletions of relatively large segments of DNA. Therefore, it seems reasonable to apply cell response models that were designed to describe reproductive death to mutation frequency as well. Two general types of models are found to be consistent with the survival characteristics of plateau-phase Chinese hamster ovary (CHO) cells (Nelson et al. 1989): sublethal damage models, such as the accumulation of damage (Roesch 1978), and potentially lethal damage models, which include radiation-induced misrepair or fixation processes (Curtis 1986). The significant difference in these models is that the latter type assumes that all repair is repair of potentially lethal damage (damage that is lethal unless repaired); the former type assumes that repair of sublethal damage (damage that by itself is not lethal, but can become lethal by interacting with additional damage) is a distinct process.

Analysis of the split-dose and delayed-plating data for HPRT mutation (Morgan et al. 1989) shows a distinct difference in these two endpoints. Cell survival experiments always show an increase in survival with increasing delay between irradiation and replating, as well as with increasing interval between doses in a split-dose experiment. However, the mutation experiments show a decrease in the

effect (mutation frequency) when the dose is split, but not with a plating delay. A logical interpretation of this phenomenon is that sublethal and potentially lethal damage repair are two distinct processes, and that the type of deletions that are repaired by the potentially lethal damage repair processes are not observed in the HPRT assay. This would be the case if those processes were repairing damage that would otherwise lead to terminal deletions or other very large deletions, such as deletions that always contain essential genes in the vicinity of HPRT. Another possibility is that only a potentially lethal damage repair process is functioning under these circumstances and that all possible repair is completed before the mutagenic damage is fixed, even with immediate plating. In order to accommodate a delayed-plating effect for survival but not for mutation, damage leading to mutations would have to be repaired faster than that leading to reproductive death.

Clearly, there is much that isn't understood about the relationship between specific mutations and reproductive death. Additional experiments, possibly with other systems capable of detecting much larger deletions, may be needed in order to detect the relationship between deletion size and the probability of repair in a delayed-plating experiment. Repair rate experiments intended to compare the effects of deletion length with those of other characteristics, which might distinguish deletions leading to viable mutants from those leading to reproductively dead cells, will also require a mutation system that shows a delayed-plating effect. Experiments involving the use of repair inhibitors may also be able to contribute in this area. Additional cell systems and endpoints that might provide these data are currently being investigated.

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Mechanisms of Radiation Mutagenesis: Nonrandom Distribution of X-Ray-Induced Mutations

T. L. Morgan, E. W. Fleck, and J. H. Miller

The biological effects of ionizing radiation are the result of a complex process leading from energy deposition through repair of damage and replication of cells to the observed end effect. Models of cell response are often used to relate characteristics of the incident radiation, such as linear energy transfer (LET) and dose rate, to the observable effect, typically cell reproductive death. However, many assumptions that cannot be tested individually have to be made in order to build these models. The number of assumptions in a model can be reduced if it describes an endpoint that involves less processing of the damage by the biological system. Mutation is one of these endpoints. The specific changes in the DNA of a gene that has been mutated can be investigated using molecular techniques. The molecular characteristics of x-ray-induced mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells are currently being investigated. As reported previously (Miller et al. 1989), the most frequently observed lesion was caused by a complete

deletion of this gene. Almost two-thirds (54 out of 89) of the cell lines tested showed a complete loss of HPRT-specific sequences as determined by Southern blot analysis (Southern 1975) using a cDNA copy of the hamster HPRT gene as a probe. The remaining 35 cell lines showed evidence of less substantial changes: partial deletions, rearrangements, or small, localized lesions such as point mutations. Fifteen of these cell lines presented patterns consistent with a partial deletion of the HPRT locus. The banding patterns of these cell lines were compared with a restriction site map of the Chinese hamster HPRT locus (Rossiter 1987) with the intention of determining the approximate size and location of each lesion. For the purposes of this analysis, each lesion was assumed to have arose as the result of a single mutational event. With knowledge of the exon complement of each restriction fragment, the deletion breakpoints were localized to specific regions of the locus for each of these 15 cell lines. Figure 1 shows the approximate size and location of the lesions in these 15 cell lines. Intragenic deletions ranging in size from approximately 5 kbp to 13 kbp were observed in 9 mutants (3F3, 6I, 10E, 12A, 12D, 13D, 13E, 4SF, 4PA). Two mutants suffered substantial deletions on the 3' side of the locus (8B and 8C); three other mutants were caused by loss of sequences in the 5' half (2SB, 4PC, 2SF). One mutant, 10G, resulted from a deletion extending from the 5' flanking region to a point between exons 6 and 7. The deletion size could not be estimated in these latter six mutants because extension of the deletion into flanking regions could not be ruled out. The pattern of deletions represented in Figure 1 does not appear to be random. Although the size of most of the deletions could not be determined precisely, there appears to be a disproportionate number of mutations in the 3' half of the gene (i.e., between exons 4 and 9). These data suggest that this region of the hamster HPRT gene is abnormally sensitive to radiation-induced events creating deletion-type mutations.

Ten other mutants presented banding patterns that were not consistent with deletions of the type shown in Figure 1. In most cases, interpretation of the banding patterns from several different enzymes resulted in conflicting conclusions regarding the nature and extent of the lesion (data not shown). Two mutants (12B and 12E) showed altered patterns consistent with the presence of

FIGURE 1. Structure of the Chinese Hamster HPRT Gene and Position of Deletion Breakpoints in Mutants 2SB, 3F3, 8B, 4PC, 8C, 2SF, 10E, 4SF, 13D, 12A, 12D, 13E, 4PA, 10G, and 6I. Exons 2 to 6 and 9 are located as indicated. Exons 7 and 8 have been localized to the region within the stippled box. Black bars indicate deletions. Stippled bars represent uncertainty in the position and/or extent of the deletion. The approximate location of four mutations of unknown type (i.e., point mutation, insertion or deletion) are indicated by open bars containing question marks.

deletions when one restriction enzyme (Ssp I) was used. However, the banding pattern observed with two other enzymes (Pst I and Hind III) remained unchanged. These mutants were included in the alteration-type mutation class, but it could not be determined where the mutation was located because exon assignments for the Ssp I bands have not been determined. Four other mutants showed pattern changes consistent with deletions when one enzyme was used. However, when a different enzyme was used, no changes were detected. These data suggest the presence of small deletions or point mutations that either remove a restriction site or generate a new site. By this analysis, one of these four mutants (6H) appeared to have a small lesion in the 3' half of the gene; the lesions in the remaining three mutants (2SC, 4DA, 4DH) appeared to be located in the 5' side. The approximate location of the lesions in these four cell lines is shown in Figure 1. Banding

patterns of the four remaining mutants (6G, 10H, 13A, 4SB3) were too complex to interpret, suggesting the presence of chromosomal rearrangements such as translocations or inversions.

It is generally believed that the spatial pattern of radiation energy deposition is random and hence should lead to a similar random distribution of genetic lesions. The data presented in Figure 1 indicate that this assumption is incorrect. It appears that the 3' half of the hamster HPRT gene is abnormally sensitive to the mutagenic effects of 250-kVp x-rays. A similar pattern of clustering has been observed in mutant Chinese hamster V79 cells in mutants isolated from cell populations treated with either γ -rays or α -particles.^(a) The current work as well as work presented by Thacker et al.^(a) represents the largest studies conducted to date on the location of radiation-induced deletion breakpoints. Previous, more limited studies have suggested a similar conclusion. Groszovsky et al. (1986) irradiated CHO cells with γ -rays and characterized 55 APRT (adenine phosphoribosyltransferase) mutants. Three were determined to be intragenic deletions and may have had identical breakpoints. Pastink et al. (1988) analyzed five x-ray-induced deletions at the *white* locus of *Drosophila melanogaster* and found that they were nonrandomly distributed: two contained the same 29 bp deletion, and 3 others were clustered. Spontaneous mutations have also revealed an unexpected clustering of deletion breakpoints. In an extensive study of mutations in the HPRT locus of human B-lymphoblasts, Gennett and Thilly (1988) observed that a disproportionate number of deletion endpoints occurred within a restricted region of the gene: 16 of 27 breakpoints were found to lie between exons 4 and 7. In agreement with our investigations, the density of deletion breakpoints within this region of the human HPRT gene was more than twice as high as could be expected on the basis of a random distribution.

Taken together, PNL's results as well as results from other laboratories indicate that certain regions of the mammalian genome may be uniquely

(a) J. Thacker, E. W. Fleck, B.J.F. Rossiter, and T. L. Morgan. "Localization of Deletion Breakpoints in Radiation-Induced Mutants of the HPRT Gene in Hamster Cells." Submitted to Mutation Research.

sensitive to mutations occurring spontaneously or induced by ionizing radiation. This conclusion will have important implications for future biophysical modeling efforts. For example, such a model predicts that how a cell repairs or otherwise processes radiation damage will have a much greater effect on cell survival than the specific location of the damage itself.

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Radiation Biophysics

In the Radiation Biophysics project, specific radiobiological studies are conducted to test various aspects of the mathematical models developed in the Modeling Cellular Response Program and compare these with models explored in Radiation Dosimetry. These studies are designed to determine whether specific mathematical expressions, intended to characterize and describe the expected effects of biochemical mechanisms on cellular response, are consistent with the behavior observed in selected biological systems. Cultured cells are used for these experiments. Since stringent requirements are usually placed on the cellular system, special techniques and culture conditions are used to minimize biological variability. For example, cells that have ceased progression through the cell cycle are required to study the effects of dose protraction during long-interval split-dose or low-dose-rate studies. In some cases, drugs are used to control the response of cells, and in others, molecular biology techniques are used to measure alterations in the cells that have not been expressed as cell death.

The Total Amount of Damage Repaired by Plateau-Phase CHO Cells

J. M. Nelson and L. A. Braby

A series of experiments have been initiated using the repair inhibitor adenine-9 β -D-arabino-furano-side (β -ara-A) to determine cell survival in the absence of repair. Different assumptions regarding the consequences of unrepaired damage remaining within irradiated mammalian cells lead to different predictions of the effects of low-dose and low-dose-rate irradiation. These assumptions can be tested using split-dose and delayed-plating irradiation techniques. However, the interpretation of such experiments requires that we know the total amount of damage available to the repair machinery and capable of being observed in each of these two types of experiments. Recent results from other laboratories suggest that the change in survival following a plating delay cannot be used as an indication of the initial amount of damage since an unknown amount of repair occurs even when the plating delay or the time between dose fractions is reduced to a minimum. A comparison of split-dose and delayed-plating survival with survival following immediate plating in the presence of β -ara-A will provide the needed data to determine the total amount of repair.

As discussed previously (Nelson et al. 1989), a comparison of the survivals of plateau-phase Chinese hamster ovary (CHO) cells exposed to low doses of x-rays in split-dose and in delayed-plating experiments consistently shows higher

survival following the split-dose procedure. This result alone demonstrates that the probability of reproductive death of these cells is a nonlinear function of the concentration of damage and that much of this damage is removed both during an interval between two doses and in the interval after exposure but before replating in medium that re-initiates cell growth. Thus these results are consistent with the underlying assumptions of models, such as the dual radiation action model (Kellerer and Rossi 1972) and Roesch's accumulation of damage model (Roesch 1978), both of which assume that some radiation products (sublesions) must interact to produce a condition that is lethal or potentially lethal to the cell. These results are also consistent with models such as the lethal-potentially lethal model (Curtis 1986), which assumes that all relevant damage is potentially lethal, and that these potentially lethal lesions interact in some way to produce irreparable lesions. The differences between these two types of models are significant since they result in different extrapolations from high-dose and high-dose-rate data to effects at low doses and low dose rates. In the potentially lethal damage models, repair remains effective to the lowest dose, since even a single potentially lethal lesion can be repaired. However, in sublethal-damage models, because the probability of two sublesions interacting to produce a lethal lesion approaches zero at very low doses, the repair of sublesions is of little or no consequence. Repair occurs at these low doses, but it does not produce an observable effect on cell survival because unrepaired damage of that type is not lethal.

An experimental determination of the role of repair at low doses should have application for determining the appropriate extrapolation from experimental observations at higher doses. Additionally, it should help improve our understanding of the biochemical mechanisms of radiation damage by clarifying the role of repair or misrepair in the production of lethal damage and possibly in the production of major DNA deletions. However, two characteristics of the response of mammalian cells make the experiment relatively difficult. The existence of a delayed-plating effect demonstrates that repair of potentially lethal damage occurs, regardless of whether or not some or all of the initial damage is sublethal. Thus, the effect of repair would not be expected to completely disappear at low doses; only repair of sublethal damage (that portion of repair involved in the alteration of damage requiring interaction to become lethal) would be eliminated. As a result, the statistical fluctuations inherent in any biological experiment make it impossible to rule out the existence of any sublethal damage. This permits determination of only an upper limit for the fraction of the initial damage that could have been sublethal. Thus, very large numbers of cells must be used to reduce experimental uncertainties. Such experiments are highly desirable. If it could be shown that the majority of the damage is potentially lethal, the sublethal component could be ignored without incurring significant errors in risk estimates when extrapolating from high-dose data and without neglecting significant biochemical mechanisms.

The other complicating or limiting characteristic of the biological system for such measurements is the delay between trypsinization and the actual time at which remaining damage becomes fixed. This delay has been observed in many cell systems; it may be as long as a few hours in some cases. As a consequence, this could prevent the first part of the repair from being detected in a delayed-plating experiment. In our system, most of the damage is repaired within 1 h of trypsinization. If the first part of the repair cannot be detected, delayed-plating experiments probably would not reflect the initial amount of damage. Without knowing the initial amount of damage, it is not possible to compare the amounts of damage repaired as a function of dose.

Techniques for the use of the repair inhibitor β -ara-A have been developed (Iliakis et al. 1986). PNL has modified these to study the total amount of repair occurring under various post-irradiation (delayed-plating) treatments. To have the drug present immediately after exposure, the cells are trypsinized and suspended using solutions containing 50- μ M β -ara-A, and the subcultured cells are incubated for 4 h in growth medium containing β -ara-A. After 4 h, the medium containing β -ara-A is aspirated and the cultures are refed with drug-free medium. A substantially lower plating efficiency, on the order of 40 to 50% compared to 70 to 80% for untreated control cells, results from the toxic effects of both the β -ara-A and the acid diluent into which it must be dissolved. It is not possible to exclude the acid effect since the material is insoluble at or near a neutral pH; however, a correction factor for each can be included in the evaluation of the survival for repair.

Twelve experiments, each involving 30 irradiated or control plates and yielding nearly 400 survival assay plates (about 100,000 surviving cells) have been performed. Several of these were done to compare survival of cells plated immediately after irradiation, with and without β -ara-A, to survival when plated 24 h after irradiation without β -ara-A. Radiation doses of 0.50 to 2.50 Gy were given to these cells. Other experiments also included split-dose survival measurements for comparison with previous experiments. In all cases, surviving clones are counted using an automated video-analysis system. Results of these experiments are presently being analyzed to determine the total amount of damage produced and remaining in the absence of repair as a function of dose.

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Frequency on DNA Breaks in a Specific Gene

J. M. Nelson and T. L. Morgan

A variety of recent experimental results suggests that the radiation-induced damage in DNA that leads to observable end effects, such as cell death or mutation, is not randomly distributed. Madhani et al. (1986) have shown that repair occurs more rapidly on actively transcribing genes than in inactive ones. Active genes are nonrandomly distributed throughout the chromatin. PNL has shown that the ends of deletions that cause hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutations in Chinese hamster ovary (CHO) cells are also not distributed randomly throughout the gene.

Since there is no reason to believe that charged particle tracks through the nucleus of a cell are not random (with respect to chromosomes and other nuclear structures), this nonrandom distribution of observed damage must result from interaction between the initial products of energy deposition and the biological target, or from the modification of that initial damage by biological processes within the cell. If interaction is involved, the actual nature of such interactions is unknown. This differential effect may occur early, possibly as a result of "hot spots" in the DNA where common radiation products are more likely to produce strand breaks or some other irreparable damage. It may also be effective later in the processing of

damage, as the result of local conditions or characteristics of the chromatin that inhibit repair of some commonly occurring damage.

In order to investigate the processes leading to the formation of the large deletions that are characteristic of radiation-induced mutations, it is necessary to detect the damage at various stages in its processing. The damage must be studied as it is modified by various biochemical mechanisms within the cell, and the frequency of damage remaining in relatively small regions of the chromatin must be related, if possible, to differences in physical or chemical structures that characterize in those regions.

In order to investigate the origin of this nonuniform distribution of deletion endpoints, a technique has been developed for measuring the frequency of strand breaks in a single gene within a population of mammalian cells. Several million cells are lysed in situ and their DNA extracted by conventional phenol-chloroform extraction methods. This DNA is then digested overnight with infrequent cutting enzymes such as Sfi I and BssH II. After ethanol precipitation and resuspension in a suitable buffer, the cut fragments are separated electrophoretically on a low-density agarose gel. The separated DNA is then denatured and transferred to a membrane to be probed with labeled DNA made specifically to the gene of interest. With the proper restriction enzymes and a suitable probe, the gene of interest can be found as a band or bands of labeled DNA. These bands are well-defined, reflecting specific fragment lengths as a result of cutting the DNA at the recognition sequences characteristic of the enzymes used. In irradiated cells, there is a dose-dependent probability of additional double-strand breaks in that gene. When an additional break has occurred, two smaller fragments result and the DNA content of the characteristic band will be reduced because shorter fragments will have migrated further through the gel. The well-defined bands then give way to smeared bands.

Scanning densitometry of autoradiographs is being used to evaluate the DNA content in the band as a function of dose. This approach works well, but further refinements in the processing of the probe and the quantitative measurement of the DNA content of a band are needed to adequately resolve the small differences in the dose-response

relationship as a function of gene structure or transcriptional activity. Initial experiments, now underway, will investigate the extent of repair in different genes following doses that are high enough to produce at least one break in the gene in almost every cell.

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Modeling Cellular Response to Genetic Damage

The objective of this project is to understand the mechanisms that determine cellular responses to physical and chemical carcinogens with particular emphasis on the role of chromosomal damage in cell killing and mutation induction. PNL's approach combines kinetic modeling of cellular response with molecular modeling of the interactions of radiation and chemicals with DNA in its cellular environment. Currently the focus is on the mechanisms and effects of interaction between energy deposition events in cells exposed to ionizing radiation. Monte Carlo simulation of the slowing down of charged particles in biologically significant materials is the primary tool for investigating these effects at the molecular level. Models of energy deposition by neutrons in oriented DNA fibers suggest that intramolecular energy and/or charge transfer allows events initially separated by several hundred base pairs on the same DNA chain to interact in ways that influence free-radical yields. At the cellular level, interactions between energy deposition events are believed to affect the dose and dose-rate dependence of cell killing and mutation induction through their influence on the cell's ability to repair radiation-induced damage.

Role of Energy and Charge Transfer in DNA Damage by Densely Ionizing Radiation

J. H. Miller, W. E. Wilson, and C. E. Swenberg^(a)

The biological effectiveness of densely ionizing radiation relative to x-rays is an important consideration for radiation safety in many terrestrial and space environments. The mechanisms that cause radiations with high linear energy transfer (LET) to be more effective per unit of dose in producing lethal, mutagenic, and carcinogenic effects are poorly understood; however, they are generally believed to be related to the patterns of energy deposited in critical subcellular targets like the nucleic acids. PNL is investigating the role of macromolecular structure in radiation-induced DNA damage through extension of the methods that were developed to predict the effects of track structure on radical production in simple homogeneous materials (Miller and Wilson 1989).

In most highly disordered molecular liquids and solids (e.g., water), inelastic mean free paths (IMFP) of particles in a radiation field are the main determinant of energy deposition patterns. In polymeric materials, high disorder does not necessarily imply immediate localization of energy absorbed from a radiation field. Absorption of energy may involve excited states in which energy and/or separated charges are highly mobile. When this is the case, the distribution of traps for energy

quanta and charge in the material before radiation exposure may become as important as IMFP in determining the spatial distribution of damage induced in the irradiated sample.

Under physiological conditions, DNA exhibits several modes of intramolecular energy and charge transfer that may have sufficient range to influence the spatial patterns of radiation-induced damage. One of these is the so-called "breathing mode" of vibrational excitation. In this mode, a disruption of base pairing between complementary strands moves along the DNA chain at approximately the speed of sound. This transient melting of the double helix is believed to be important in the initiation of transcription. Another important characteristic of DNA is its ability to organize water of hydration in order to assume physiological significant conformations. van Lith and coworkers (1986) have recently shown that electrons ejected from valence orbitals by absorption of energy from ionizing radiation can enter conduction-band-like states of hydrated macromolecules where they have high mobility and a long lifetime.

PNL is investigating the effects of these long-range modes of energy and charge transfer on the production of free radicals by protons slowing down in oriented DNA samples. The relationship between data on neutron-induced free radicals (Arroyo et al. 1986) and mobile electronic and vibrational states of DNA was discussed in a recent publication in the *International Journal of Radiation Biology* (Swenberg and Miller 1989). Model calculations (Miller et al. 1988a and 1988b) suggest that energy and/or charge must migrate

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over hundreds of base pairs in order to explain the dependence of radical yields on the orientation of DNA chains relative to the neutron flux. With the help of exploratory research funds, experimental methods to test these models by direct proton irradiation of oriented DNA samples are also being developed.

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Effect of DNA Sequence and Structure on Radiation Sensitivity

J. M. Nelson, M. Ye, and J. H. Miller

Observations of preferential sites for termination of deletion mutations is but one of many lines of evidence that suggest that some regions of DNA are more sensitive to radiation damage than are others. DNA sequence and conformation may be a part of the mechanism responsible for these variations. Studies of ϕ X174 phage DNA by Nabben et al. (1984) indicate that the double-stranded conformation is about one-fifth as sensitive to strand scission as the single-stranded form of ϕ X174 DNA. Since torsional stress tends to confer

single-stranded character to A T-rich regions of DNA, greater yields of strand breaks may be a part of the mechanism for the enhanced radiation sensitivity of active genes (Chiu et al. 1982), which exhibit greater torsional stress than the bulk of mammalian chromatin. To test this hypothesis, PNL is attempting to measure the frequency of strand breaks under different conditions of superhelicity, using several phage and plasmid systems.

To study the role of variations in torsional stress, several topoisomers of pBI 30 (provided by Charles Swenberg of Armed Forces Radiobiology Research Institute) have been irradiated with 250-kV x-rays at 0°C and the resulting fragments have been separated by agarose gel electrophoresis. The fraction of the DNA remaining in Form I (closed, superhelical plasmid) decreases exponentially with increasing dose from 0 to 1200 Gy due to radiation-induced strand breaks. The fraction of Form II (relaxed plasmid with one strand broken) increases correspondingly. Furthermore, at each dose, the fraction converted to Form II is greatest for the plasmids with the highest degree of supercoiling, which is consistent with the hypothesis that torsional stress is conferring single-stranded radiation sensitivity to the plasmid. However, it is not always observed that radiation sensitivity is a monotonically increasing function of supercoiling, which suggests that other mechanisms may be involved in strand scission. PNL is currently improving experimental techniques by scanning the gel directly with a fluorimeter rather than attempting to quantitate fluorescence intensity from a photograph of the gel being exposed to ultraviolet light. When the measurement technique has been refined satisfactorily, additional measurements will be made to determine the quantitative relationship between torsional stress and strand breakage. Then, systems such as the plasmid pRRR will be irradiated to study the relative probability of strand breakage in the A T-rich and G C-rich regions of these systems.

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Interaction of Proton Tracks with Oriented DNA Molecules

L. A. Braby and J. H. Miller

The biological effectiveness of high linear energy transfer (LET) radiations may depend in part on intramolecular energy transfer in DNA. Discretionary funds are being used to develop experimental techniques to test predictions of models for the effect of energy and charge transfer on the yield of free radicals produced by high-LET radiation. One of the predictions of these models is that when a sample of oriented DNA is irradiated, the yield of 5,6-dihydrothymine-5-yl (TH) radicals will depend on the direction of the high-LET charged particle tracks relative to the DNA molecules. This type of effect was observed when oriented DNA fibers were irradiated by neutrons at liquid nitrogen temperature (Arroyo et al. 1986). However, secondary particles produced by such a beam are not highly collimated, and the use of neutrons cannot provide a definitive test of the model. A better approach is to use a collimated beam of monoenergetic protons produced by a charged particle accelerator. PNL has designed and set up such a system to irradiate small rectangular samples of oriented DNA fiber in vacuum at liquid nitrogen temperature. The sample is mounted on the beam stop of a faraday cup, covered with a thin piece of mylar to prevent changes in the hydration state of

the DNA, and placed in the accelerator beam line. The sample is cooled to 77K by liquid nitrogen flowing through a passage in the beam stop, and the beam line is then evacuated. The DNA sample is exposed to a proton beam of selected energy between 2 and 4 MeV. The proton fluence and dose are calculated from the charge collected on the faraday cup and the area of its entrance aperture.

Oriented DNA samples with the fibers either parallel to the irradiated surface or perpendicular to it have been obtained from Professor Rupperecht at the University of Stockholm. Pilot studies have been done using these samples to test the irradiation system and the electron spin resonance (ESR) equipment used to detect the radicals. As a result of these tests, a number of refinements in the sample cooling and transfer system have been made. Preliminary data indicate that unlike the results of Arroyo et al. (1986), some TH radicals are produced when the proton flux is perpendicular to the DNA orientation. To better quantitate this finding, PNL has modified the ESR spectrometer to obtain data in digital form. These data, which indicate a mixture of radicals after irradiation, will be analyzed by computer simulation to estimate the yield of TH for comparison with model predictions.

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